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BRAIN METABOLISM DURING ELECTRONARCOSIS[1]

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In mammals a state resembling narcosis has been obtained by the application of electrical currents to the brain through electrodes located on the outside of the head. This state, electronarcosis (en.), can be produced by currents with very different properties; unidirectional pulse current (Leduc, 1902), pulse current with alternating direction of the pulses (Nagelschmidt, 1912), 50 cycle sinusoidal alternating current (van Harreveld and Kok, 1934). It was recently shown in this laboratory that the electronarcotic effect is due to the stimulative action of the current applied (van Harreveld, Plesset and Wiersma, 1942). This fact indicates that the mechanism of en. differs from that of chemical narcosis, even though the symptoms of the two states may resemble each other very closely.

Alexander and Cserna (1913) found that ether narcosis causes a severe depression of the brain metabolism. Administration of morphine or magnesium sulfate also decreased the oxygen consumption of the brain. This was confirmed by Gayda (1914). Because of the differences in mechanism of chemical and electronarcosis it seemed of interest to determine the brain metabolism during en.

METHODS AND RESULTS. The metabolism of the brain before, during and after en. was determined by measuring the decrease in oxygen content of the blood during its passage through the central nervous tissue (A-V difference), and by measuring the amount of blood flowing through the brain. For technical reasons it was found impractical to make both of these determinations simultaneously; the blood flow experiments require considerable preparation, making chemical narcosis immediately before the experiment necessary, and since this interferes with the oxygen uptake of the brain, the changes in A-V difference were determined in experiments in which no general narcotic was used.

En. was given as described before (van Harreveld, Plesset and Wiersma, 1942).

[1] This investigation was supported in part by a grant from the Department of Institutions of the State of California.

[2] Hixon Fellow.

Two electrodes were placed on the temples directly behind the eyes. En. was initiated with 250–300 ma. 60 cycle alternating current. After 30 sec. the current was decreased to a level where respiration returned. With this current the animals could be kept in en. for any time desired.

Dogs were used in all the experiments.

1. *Determination of blood flow in the brain and its relation to blood pressure.* Rein's method of blood flow determination, in which heating electrodes and thermocouples are placed directly in contact with the artery, could not be used in the present experiments since the initiation of en. is characterized by strong convulsions which would result in movements of the electrodes with respect to the vessels. Therefore the combined blood streams of the central ends of the severed carotids were led through flexible tubes to a flow meter placed outside the body and then through a heating coil, back to the peripheral ends of these arteries. Before re-entering the body the blood temperature was measured, and the temperature of the water round the coil was adjusted as to heat the blood to about 38°.

The flow meter used was built on the Rein principle and consisted of a thick walled lucite tube (5 mm. inner diameter) through which the blood stream passed. In the middle of the tube the blood passed between two heating electrodes. These electrodes were 2.5 mm. apart and their shape, size and placement in the canal were such that the blood stream could be heated uniformly by a 60 cycle heating current passing through the blood. The power used was 2 to 3 watts. The temperature difference of the blood before and after passing between the heating electrodes was measured with two thermocouples and a galvanometer. The flow meter was calibrated with defibrinated ox blood. A practically linear relation was found between the blood flow and the reciprocal of the galvanometer deflection per watt of heating power.

Under ether narcosis the thyroid and external carotid arteries were ligated. In some experiments the vertebral arteries were left untouched; in most they were clamped off. In the latter cases care was taken to supply the brain with blood throughout the manipulations, by connecting first one carotid with the flow meter and to connect the other only after the circulation through the first had been re-established. Blood pressure was recorded from the femoral artery. Fastusol B.B.A., 50 mgm./kgm. body weight (Modell, 1939) and heparin[3], 5 mgm./kgm. body weight were used as anticoagulants.

a. *Effect on blood pressure* (fig. 1, broken line). The variations in blood pressure before en. can usually be traced to stimulations of the animal during this period. Immediately after applying the current, a drop in blood pressure occurs. The extent of this drop is rather variable. After a few seconds, however, the pressure rises above the prenarcotic value. This rise usually lasts for several minutes after the initial high en. current has been reduced. The blood pressure eventually falls off to about the level found during the prenarcotic period. These changes are in complete agreement with the observations made by Bikeles and Zbyszewski (1920), Ivy and Barry (1932), Roos and Koopmans

[3] The heparin was kindly supplied by Hoffman-La Roche, Inc.

(1934) and by van Harreveld and Kok (1934). After the termination of en. a sudden drop in blood pressure is often observed.

b. *Effect on blood flow* (fig. 1, solid line). The data for the calculation of the blood flow (deflection of the galvanometer, voltage and amperage of the heating current) were recorded once a minute. In all experiments the blood flow had a tendency to decrease slowly and continually during en. as well as during the control periods before and after current application. This decline is not caused by changes in the blood pressure as in many cases the mean pressure does not change materially during the entire experiment, except during the short period immediately after the onset of en. Immediately after the start of en. large changes in blood flow occur which parallel the blood pressure variations. During the first seconds of current application, the blood flow drops. This is followed by a sharp rise which often raises the blood flow for a few minutes high above the prenarcotic value. In the next few minutes the flow drops again

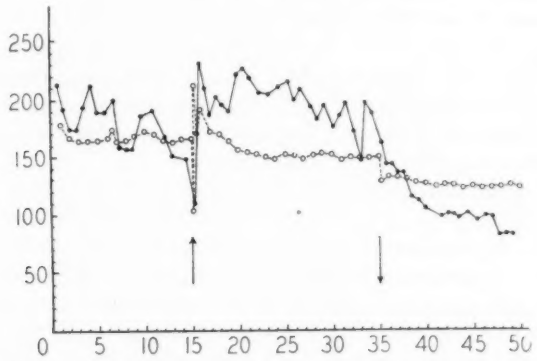

Fig. 1. Blood flow (solid line) and blood pressure (broken line) during a blood flow experiment. On the abscissa is plotted the time in minutes, on the ordinate the blood flow in centimeters per minute and the blood pressure in millimeters Hg. At the arrow pointing up electronarcosis is started, at the arrow pointing down the current is cut.

somewhat. From then on the blood flow continues to decline slowly during the en. period. In this way the blood flow, during the main part of en., is always larger than would be expected from its prenarcotic slope. When the en. current is cut, the flow often drops sharply. A good demonstration of this phenomenon can be seen in figure 1. In this experiment the blood pressure also shows a slight drop at the end of en. The drop in blood flow, however, continues long after the blood pressure has reached its new lower level.

It thus seems that en. raises the level of blood flow without interrupting the continuous decline observed during the control periods before and after en. There is a considerable variation in the rapidity of the decline in different experiments; sometimes the blood flow after 20–30 min. of en. is still well above its prenarcotic level. In other experiments it dropped considerably below this level notwithstanding the rise caused by en.

In some of the control experiments in which the animal was prepared as usual

but in which no en. was given, the same gradual decline of the blood flow was found without, of course, the increase due to en. In other controls, however, the blood flow dropped only for about the first 30 min. but then leveled off. The decline is not a specific effect due to the administration of fastusol since a drop of similar magnitude was found when heparin was used as anticoagulant.

In the experiment given in figure 1, the vertebral arteries were clamped off. A similar picture is obtained when the vertebral arteries are left intact. In the latter experiments the total amount of flow measured through the internal carotids is smaller.

2. *Determination of A-V differences.* On the day before the experiment a $\frac{1}{2}$ inch hole was trephined in the skull over the sagittal sinus. This was performed under nembutal-ether narcosis and with aseptic precautions. Into this hole a threaded piece of brass tubing $\frac{1}{2}$ inch high and $\frac{1}{2}$ inch in diameter was screwed. The spinal cord was transected in the lower thoracic region to make possible recording of the blood pressure from the femoral artery during the actual experiment without the use of a general anesthetic.

The next day the dura, at the bottom of the tube, and the surrounding skin were anesthetized with novocain, and a femoral artery was prepared and connected with a recording manometer. The manometer connections and the cannula were filled with 5 per cent fastusol solution and 50 mgm. of the dye per kgm. bodyweight was injected intravenously.

The venous samples were taken in the following manner: the brass tube was filled with thin mineral oil, then the sinus was punctured and the blood accumulating under the oil was sucked up in a syringe partly filled with oil. The tube was then closely packed with cotton until the next sampling. Arterial samples were obtained from the intact femoral artery. The samples (5 cc.) were transferred under oil to tubes cooled by ice water until the time of the oxygen determination. It was found that blood can be kept for several hours under these conditions without significant changes in the oxygen content. The oxygen content of the samples was determined by the method of van Slyke and Neill (1924).

In most experiments 6 sets of venous and arterial samples were drawn, namely, two controls, about 20 and 5 min. before the start of en.; two sets during en. 10 to 15 and 25 to 30 min. after the start; and two more controls 10 to 15 and 25 to 35 min. after the end of en. The first sample during en. cannot be taken earlier than about 10 min. after the start because, as has been shown above, rapid and large changes in blood flow occur during the initial phases of en. Besides there is a complete respiratory arrest during the first minute of en.

In table 1 the oxygen A-V differences of 7 experiments are given. The overall values show that the A-V differences increase notably during the experiment. This tendency is present in the period before, during and after en., as a comparison of the two values obtained during each of these periods shows. The effect of en. is to depress the A-V differences without interrupting the slow increase. Thus the beginning of en. causes a drop of the A-V differences, whereas a notable increase of this value is observed after the end of en. The individual experiments show considerable deviations from this average. Especially it should be

noted that in some experiments the A-V differences during en. instead of being smaller are larger than before current application.

Discussion. It is likely that the slow decline in blood flow observed is due to a number of factors among which the cessation of ether narcosis, the use of

TABLE 1

A-V differences during electronarcosis experiments

Each observation consists of three figures. The upper one indicates the time in minutes before the start of en. (columns 1 and 2), after the beginning of en. (columns 3 and 4), and after cutting the current (columns 5 and 6). The middle of the three figures is the A-V difference in volume per cent at these moments, and the lower figure is the blood pressure in millimeters Hg at the moment the sample was taken.

NUMBER	BEFORE ELECTRONARCOSIS		DURING ELECTRONARCOSIS		AFTER ELECTRONARCOSIS	
1	20′ 13.3 % 103 mm.	5′ 13.0 % 117 mm.	15′ 8.8 % 95 mm.	30′ 11.5 % 102 mm.	15′ 16.1 % 100 mm.	30′ 16.6 % 86 mm.
2	22′ 9.8 % 99 mm.	7′ 9.8 % 98 mm.	11′ 11.5 % 85 mm.	28′ 13.5 % 98 mm.	10′ 13.6 % 79 mm.	
3	18′ 14.8 % 134 mm.	6′ 16.3 % 123 mm.	10′ 17.9 % 123 mm.	26′ 19.6 % 139 mm.	12′ 19.1 % 124 mm.	25′ 19.9 % 100 mm.
4	21′ 11.8 %	5′ 12.8 %	12′ 12.8 %	28′ 12.3 %		35′ 17.2 %
5	20′ 13.3 % 128 mm.	5′ 15.1 % 132 mm.	15′ 13.3 % 159 mm.	30′ 11.8 % 148 mm.	16′ 13.5 % 105 mm.	35′ 12.3 % 90 mm.
6	19′ 13.4 % 96 mm.	4′ 15.1 % 123 mm.	16′ 12.2 % 98 mm.	31′ 12.9 % 109 mm.	15′ 15.3 % 100 mm.	30′ 15.1 % 106 mm.
7	20′ 12.6 % 80 mm.	5′ 10.5 % 118 mm.	10′ 9.3 % 108 mm.	25′ 11.8 % 124 mm.	8′ 12.6 % 92 mm.	28′ 13.5 % 94 mm.
Average A-V differences	12.7 %	13.2 %	12.3 %	13.3 %	15.0 %	15.8 %

an anticoagulant, and perhaps the application of the en. current have to be considered.

The administration of ether, necessary for the preparation of the animal for the blood flow experiments, was stopped shortly before the beginning of the blood flow recording. Alexander and Cserna (1913) and Gayda (1914) found

that the blood flow is decreased during chemical narcosis. This would exclude the possibility of explaining the slow decline of the blood flow observed in the present experiments by the cessation of ether narcosis. However, Finesinger and Cobb (1935) saw that the pial vessels are dilated during ether narcosis. These vessels contracted again when narcosis was stopped. Such a contraction, if present throughout the brain, would explain the decline of the blood flow during the first part of the experiment.

There are indications that a brain edema develops under the experimental conditions, for at the end of the blood flow experiments brain material was found to protrude through a hole made in the skull. The development of this brain edema may explain the continued slow decline in blood flow. Since Jacobi and Magnus (1925) observed the development of brain edema during en., it is possible that the application of the en. current at least facilitates the development of the edema. On the other hand, the same slow decline of blood flow throughout the experiment has been found in some of the controls. It is possible that the use of an anticoagulant has caused the edema in these cases. Since the same amount of fastusol has been injected in the blood flow and in the A-V difference experiments, the two series of experiments are comparable in this respect.

A parallel between the blood pressure and blood flow exists during the earlier stages of en. which is indicative of the close dependence of these two values. However, the mean blood pressure is not the only factor influencing the rate of flow. It was found that the blood flow level is raised for the duration of en. This is true even if the blood pressure during en. is lower than before (see fig. 1). The stimulating effect of en. on the blood flow may be due to a dilating effect on the brain vessels. This is supported by an observation of Jacobi and Magnus (1925), who found that the pial vessels during en. are markedly dilated.

Most of the A-V differences found in the present experiments on non-narcotized animals are between 12 and 16 vol. per cent. In some experiments in which A-V differences were determined shortly after stopping ether narcosis, much lower values, between 4 and 8 vol. per cent, were found. These figures are in complete agreement with Alexander and Cserna's values under the same circumstances.

The trend in the changes of the A-V differences (table 1) and of the blood flow (fig. 1) are in opposite directions. Thus, whereas en. depresses the A-V differences, it raises the level of the blood flow. Also, whereas the A-V differences gradually increase during the periods before, during and after en., the blood flow gradually declines. Thus it appears that all changes in the A-V differences found in these experiments are mainly reflections of the changes in the rate of blood flow. It is true that in some experiments (expts. 2 and 3, table 1) the A-V differences 10 to 15 min. after the start of en. were higher than before, but in these experiments the decline of the blood flow may have been so rapid that the flow has fallen below the prenarcotic value at the time the sample was taken.

Since the changes in A-V difference caused by en. seem to be mainly due to variations in the blood flow, it can be concluded that the changes in brain metabolism produced by en., if any, certainly are not large. The question whether

en. influences the brain metabolism in the same way as ether narcosis must thus be answered negatively, and the present experiments support the conclusion (van Harreveld, Plesset and Wiersma, 1942) that the nature and mechanism of electronarcosis differ widely from those of chemical narcosis.

We are indebted to Dr. M. S. Plesset, Mr. C. H. Ellis and Mr. E. B. Wright for their advice and assistance.

SUMMARY

1. The influence of electronarcosis on the blood flow through the brain has been investigated. Electronarcosis has been found to have a tendency to increase the flow.

2. In other experiments in which no general narcotic was applied, the effect of electronarcosis on the A-V differences of the brain was examined. This effect appeared to be a depression of the A-V differences.

3. A comparison of the changes of the A-V differences before, during and after electronarcosis, with the course of the blood flow during these same periods, showed that the former are mainly reflections of the latter.

4. It therefore can be concluded that electronarcosis does not cause large changes in the brain metabolism and thus that electronarcosis differs greatly from chemical narcosis in its effect on the oxygen consumption of the central nervous system.

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EFFECT OF ATROPINE ON UTERINE RESPONSES TO HYPOGASTRIC NERVE STIMULATION. ACTION AT THE GANGLIONIC SYNAPSE

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Cocaine, which generally potentiates the peripheral activity of adrenergic nerve fibers, does not augment the uterine responses to hypogastric nerve stimulation in the rabbit and monkey, as it does in the cat and rat (1). These results with cocaine might be interpreted as indicating a predominance of adrenergic elements in the hypogastric nerve of the cat and rat, and of cholinergic (postganglionic) fibers in the same nerve of the rabbit and monkey. It seemed possible that this hypothesis might be tested by studying the effect of atropine, a parasympathetic depressant, on the same uterine responses to hypogastric nerve stimulation.

Reports of previous studies on the effect of atropine on uterine responses to hypogastric nerve stimulation are limited to a few brief but authoritative statements. Röhrig (2) in 1879 observed that in the rabbit atropine did not paralyse the "motor" fibers to the uterus. This was confirmed by Langley and Anderson (3) who reported that they had injected up to 50 mgm. atropine intravenously, presumably in the rabbit. No further details of the experiment were given. Cushny (4) observed that "atropine had no effect on the uterus or on the hypogastric nerve, unless pilocarpine had been injected previously, when atropine restored the organ to its normal condition and prevented any further contractions from pilocarpine". These observations of Cushny have been "repeatedly confirmed" by Sherif (5).

The results of the present investigation have substantiated these earlier findings in regard to small doses of atropine. Large doses, on the other hand, depressed and frequently abolished the excitatory response of the uterus to hypogastric nerve stimulation, in both the rabbit and monkey. This difference, depending upon the dose of atropine given, may well explain why others have not reported any effect with atropine. Neither Röhrig nor Cushny stated the amount of atropine which they used, and although Langley and Anderson injected "up to 50 mgs.", which is admittedly a large dose, it is not easy to evaluate their statement without further experimental data. It is possible that with this large dose a reduction in response might have been unrecognizable by naked-eye observation, for Langley and Anderson did not use any mechanical recording of uterine activity.

In carrying out the experiments with large doses of atropine, we were finally led to suspect that the drug might be acting at the ganglionic synapse, rather than at the neuro-effector junction in the uterus.

METHOD. The experimental set-up for hypogastric nerve stimulation and

the recording of uterine activity were the same as previously reported (1). Seventeen experiments in rabbits and 6 in monkeys (Macaca mulatta) were carried out. The animals were anesthetized with nembutal, 0.03 gram per kgm. of body weight, injected intraperitoneally. Atropine sulphate in 0.25 per cent solution was administered intravenously in doses of 1 mgm. to 25 mgm. These doses are highly toxic but were never lethal.

In order to determine whether the effect of atropine on uterine activity and nerve stimulation varied according to the sexual cycle of the uterine musculature, castration was performed one to three weeks prior to the experiment in seven of the rabbits. Three of these animals were given intraperitoneal injections of theelin (2000 rat units) two days, and again one day, before the experiment. In two other non-castrate rabbits, progynon (2000 rat units intraperitoneally) or pregnancy urine (10 cc. intravenously) was administered a day or two prior to the experiment.

Results. There was no essential difference in the results obtained in the rabbit and in the monkey. In only three experiments was the injection of atropine followed by any excitation of the uterus; in all the others atropine had no direct effect on uterine activity.

Small doses of atropine. The excitation of uterine activity seen characteristically in the rabbit and monkey after hypogastric nerve stimulation, was unaltered in four experiments (nos. 4, 17, 18, 46) following the administration of atropine in small doses under 5 mgm. Increasing the total dosage of atropine to 8–10 mgm. during a 10 minute period, in three of these experiments, caused depression or complete inhibition of the response. In the fourth experiment (no. 4) the amount of atropine was increased up to 15.5 mgm. in a 25 minute period, without any alteration of the response.

Large doses of atropine. In three experiments (nos. 43, 44, 48) an initial dose of 6–10 mgm. was followed immediately by marked reduction or cessation of the response to hypogastric nerve stimulation. Recovery of the response in these animals (all monkeys) did not occur for one to two hours. In the remaining 16 experiments, the initial dose of atropine was 12.5 mgm. in 14 animals, and 25 mgm. in 2 others. The response to nerve stimulation was abolished in three (nos. 1, 10, 11), diminished in ten, and unaffected in three (nos. 9, 5, 60). Where the response was reduced by the initial dose, a second administration of atropine (usually 12.5 mgm. within 5–10 min.) produced a further diminution or complete inhibition (fig. 1). The dose necessary to abolish the response varied greatly and in some animals 50 mgm. were administered before complete inhibition followed. With increase in the strength of the stimulus a small response of the uterus could occasionally be reproduced. In 2 of the 3 experiments where the first dose of atropine had no effect on nerve stimulation, a second dose of 12.5 mgm. inhibited the response in one (no. 9), but not in the other (no. 5).

It is clear from these findings that, despite an occasional "negative" result, a large dose of atropine (usually 10–25 mgm.) depressed or abolished the uterine response to hypogastric nerve stimulation in both the rabbit and monkey. Depression of the response by atropine persisted for periods of $\frac{1}{2}$–2 hours, when

hypogastric nerve stimulation once more produced its customary excitatory effect on uterine activity. The occasional negative result (nos. 5, 60), where even 25 mgm. atropine failed to affect hypogastric nerve stimulation, is puzzling. Perhaps a still larger dose in these experiments might have altered the response. One of these animals was a rabbit to which 10 cc. pregnancy urine had been administered 4 days previously and the uterus was markedly active. The other was a normal monkey.

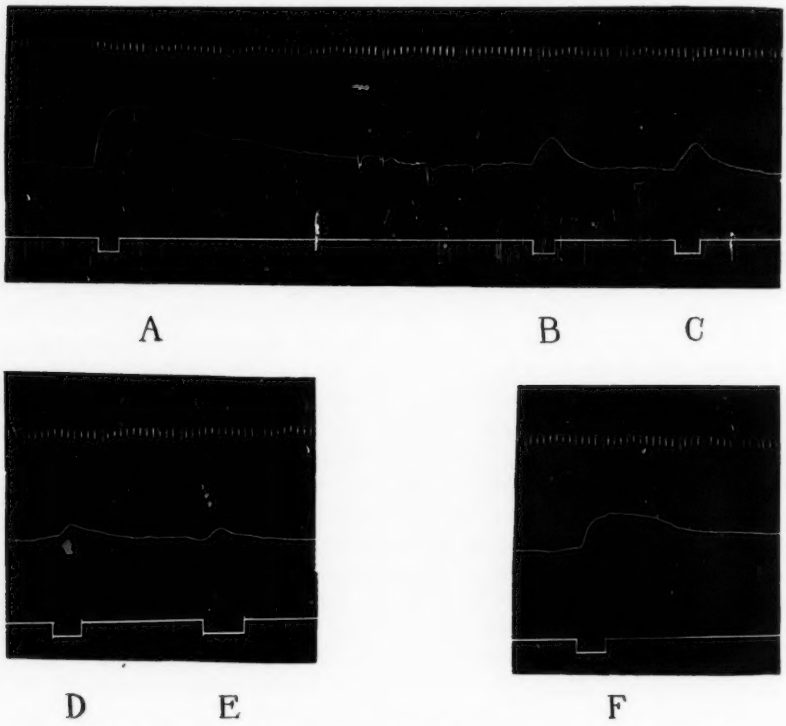

Fig. 1. Effect of large doses of atropine on the uterine response to hypogastric stimulation in the rabbit.

A B C D E F, Stim. hypogastric nerve. Between A and B atropine 12.5 mgm. intravenously. Between C and D 5 min. interval, and atropine 12.5 mgm. intravenously. Between E and F 45 min. interval. Recovery of response.

Time in minutes.

There was no alteration in the uterine response to adrenaline following the administration of atropine.

The results did not reveal any obvious correlation between the effect of atropine on nerve stimulation and the sexual cycle of the uterus. The same depression of the response by atropine was noted in normal, castrate, estrogenized, and pseudo-pregnant rabbits.

In normal monkeys the excitatory phase of the response to hypogastric nerve stimulation is frequently followed by a period of inhibition, during which rhythmic activity in the uterus is reduced to a minimum. Under certain circumstances, such as after the intravenous injection of suitable doses of nicotine, the excitatory phase is abolished but the inhibitory phase persists (6), showing that they are to some degree independent of each other. Following the administration of large doses of atropine the inhibitory phase of hypogastric nerve stimulation clearly persisted in two instances although the excitatory phase had completely disappeared. In the other experiments the inhibitory phase of hypogastric nerve stimulation was not sufficiently prominent to allow interpretation of the results.

Discussion. The dose of atropine required to abolish parasympathetic responses at the neuro-effector junction is small. In the cat 3–6 mgm. is sufficient to paralyse the chorda tympani (7) and even smaller doses have been shown to abolish the effects of vagal stimulation on the heart (8). The fact that much larger doses of atropine (10–25 mgm. and occasionally as much as 50 mgm.) were required to abolish the uterine response to hypogastric nerve stimulation would seem to suggest some action other than that at the termination of cholinergic postganglionic fibers.

There is considerable evidence to show that large doses of atropine act at the sympathetic ganglionic synapse. Langley (7), in studying the depressant action of atropine on salivary secretion found that, after 10 mgm. of atropin had been injected, the sympathetic trunk in the neck no longer could be made to cause a secretion, whilst a secretion could still be obtained from the filaments proceeding from the superior cervical ganglion. Langley did not interpret this experiment further, but others (9, 10) have confirmed the observation. The final proof as to the site of action of the drug in these experiments was given by Feldberg and Vartiainen (11). They perfused the superior cervical ganglion and observed that, although atropine in low concentrations when added to the perfusion had no effect, yet if larger doses, such as 0.1 mgm., were injected into the perfusion, a complete paralysis of response to preganglionic impulses and to acetylcholine was produced. The relatively smaller dose (2 mgm. injected into the general circulation) which Marrazzi (12) found sufficient to depress transmission through the superior cervical ganglion may be attributable to the fact that he used threshold stimulation of the cervical sympathetic trunk and a highly sensitive method of recording the effect.

Further evidence of the action of atropine at sympathetic ganglia was furnished by the following experiment, which was carried out before we became aware of the similar observation by Langley (7) on salivary secretion. The cervical sympathetic nerve in the cat was stimulated below the superior cervical ganglion, resulting in wide dilatation of the pupil and retraction of the nictitating membrane. Atropine was injected intravenously in graduated doses until a total of 12.5 mgm. was reached. At this point stimulation of the cervical sympathetic trunk below the superior cervical ganglion no longer produced any response in the eye, but stimulation *above* the superior cervical ganglion resulted

in immediate dilatation of the pupil and full retraction of the nictitating membrane.

It has been established (3, 4, 6) already that within the hypogastric nerve pathway to the uterine musculature, synaptic connections lie peripheral to the point of stimulation of the nerve, so it is theoretically possible that the effects that have been obtained with atropine are due to a depressant action at the synaptic junctions in such peripherally located ganglia. The large amounts of atropine which were found necessary to produce any effect make this interpretation probable. It is not permissible, therefore, to draw any further conclusions in regard to the nature of the postganglionic fibers within the hypogastric nerve.

The failure of small doses of atropine to depress hypogastric nerve activity cannot be interpreted necessarily as signifying that these nerves in the rabbit and monkey are not predominantly cholinergic, for there are notable examples where the activity of purely cholinergic (postganglionic) fibers is unaffected by the administration of atropine (13).

SUMMARY

1. Small doses of atropine (up to 5 mgm.) had no effect on the uterine response to hypogastric nerve stimulation in the rabbit and monkey.

2. Larger amounts of atropine (usually 10–25 mgm.) depressed and often abolished the responses for periods lasting ½ to 2 hours.

3. The results with the larger doses of atropine are probably due to the depressant action of the drug at the synaptic connections which lie along the hypogastric nerve pathway, distal to the point of stimulation.

4. Further confirmation of the action of atropine at sympathetic ganglia has been obtained by observing its effect on the changes in the pupil and nictitating membrane in response to stimulation of the cervical sympathetic below and above the superior cervical ganglion.

We are grateful to Prof. Otto Loewi for much helpful advice.

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RÔLE OF VITAMIN E IN NEUROMUSCULAR ATROPHY AND REGENERATION[1]

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Numerous investigators have observed that diets deficient in vitamin E lead to degeneration, paralysis and weakness of the skeletal muscles in various species of laboratory animals. The evidence concerning the extent to which vitamin E deficiencies are associated with lesions of the nervous system appears to be controversial and inconclusive (1, 2). Likewise, the reports concerning the efficacy of vitamin E therapy in various neuromuscular diseases have been conflicting.

This report is concerned with the rôle of vitamin E in neuromuscular atrophy and regeneration. Studies have been made concerning the effects of vitamin E-deficient diets and excess intakes of vitamin E upon the extent and velocity of denervation atrophy of muscle and subsequent neuromuscular regeneration. The experiments have been carried out on the gastrocnemius muscles and tibial nerves of guinea pigs and adult albino rats. In the former species the course of muscle degeneration resulting from deficient E intakes is extensive and rapid while in the latter the dystrophy follows a more chronic course.

EXPERIMENTAL. The mothers of young rats were placed on a vitamin E-deficient diet (3) ten days after the birth of their litter. The young were weaned at the age of 28 days and reared for 4 months on the deficient diet. The controls were animals of the same age, sex distribution and stock and were reared on an adequate diet. The members of the E-excess group received a daily supplement of 4 mgm. of alpha-tocopherol[2] per os.

Guinea pigs were placed on a standard E-deficient diet (4) and 5 mgm. of ascorbic acid per day. The group designated as "E-control" received in addition 3 mgm. of alpha-tocopherol by mouth every other day. The "E-deficient" guinea pigs received the standard E-deficient diet without alpha-tocopherol supplements. The animals in the groups designated as "E-excess" were given the basal diet supplemented with 15 mgm. of alpha-tocopherol on alternate days.

Complete denervation of the gastrocnemius was accomplished by crushing the tibial nerve at its junction with the peroneal between a heavy linen ligature and a brass rod. Such a procedure allowed for complete paralysis of the muscle and preserved good alignment for subsequent reinnervation. In certain experiments where it was desirable to eliminate muscle regeneration a section was removed from the nerve. The corresponding muscle and nerve of the unoperated contralateral limb were utilized as a control.

[1] Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

[2] The alpha-tocopherol was supplied through the courtesy of Hoffman-LaRoche, Inc., Nutley, N. J., and Merck and Company, Rahway, N. J.

At indicated times after operation, studies were made as to the strength, weight and creatine concentration in the control and experimental muscles. The muscles of some animals were utilized for histologic examination. The strength of the muscles was determined by measuring the maximal isometric tension which developed in response to volleys of supermaximal stimuli applied to the tibial nerve and directly to the muscle. The techniques employed for stimulation and muscle strength measurements have been described in detail elsewhere (5). These procedures made it possible at any chosen time to determine muscle strength, weight, creatine concentration, and the capacity of the nerve to activate its muscles; and to compare such findings with those on the unoperated contralateral control limb. Previous studies on the rat have indicated that neuromuscular regeneration runs a remarkably constant course when the animals are carefully matched as to age, body weight and sex (5).

Results. The data in tables 1 and 2 indicate that excess intakes of vitamin E had no appreciable effect upon the course of neuromuscular regeneration. Essentially the same extent of recovery of mass and strength occurred in the denervated muscles of guinea pigs and rats which had received excess intakes of E as in the denervated muscles of the control animals. The functional state of regenerating nerve, as judged by its capacity to activate muscle, was not improved by an excess of vitamin E.

The unoperated control muscles of guinea pigs and rats on vitamin E-deficient diets showed evidence of dystrophy. The creatine concentration in such muscles was found to be somewhat lower than that in muscles from control animals reared on an adequate diet. Histologic examination revealed the presence of lesions characteristic of muscle dystrophy. Functional impairment of muscle was evidenced by the finding of a weaker response per unit weight to direct and motor nerve stimulation than in muscles from control animals reared on diets adequate in vitamin E content. Furthermore, the ratio of tension elicited by nerve stimulation to that developed in response to direct muscle stimulation was considerably lower in animals deficient in vitamin E than in animals on adequate intakes. The latter finding indicates a relative ineffectiveness in the capacity of the nerve to activate its muscle and is significant in the light of a report by Telford (6) that a marked reduction occurred in the number of nerve terminals in the degenerated areas of muscles from rats on vitamin E-deficient diets.

The average values for the results of experiments on 65 rats reared on an E-deficient diet together with values for a larger number of control animals on adequate diets are presented in table 1. The results show that a diet so deficient in vitamin E as to allow the development of mild muscle dystrophy did not appreciably retard recovery from peripheral nerve injury. The loss of muscle strength and weight during a 14 day period of complete denervation was somewhat greater than that found in control animals. However, it is to be noted that in the regeneration experiments recovery of muscle mass and strength occurred at a velocity comparable to that observed in the experiments on control animals.

Studies made upon the gastrocnemius muscle of E-deficient guinea pigs 14

TABLE 1

A summary of the average values and standard errors for gastrocnemius muscles of rats on control, E-deficient and E-excess diets

CONDITION	NO. OF ANIMALS	TIME AFTER LESION	PER CENT WEIGHT LOSS†	TENSION PER GRAM MUSCLE WHEN ACTIVATED THROUGH				RELATIVE STRENGTH OF DENERVATED MUSCLE† WHEN ACTIVATED THROUGH		CREATINE IN MGM. PER 100 GRAMS MUSCLE	
				Nerve		Muscle					
				Exper.*	Control	Exper.*	Control	Nerve	Muscle	Exper.*	Control
		days									
Control....	14	12	30.5 ±1.2			1257	1878		43.8 ±1.8	340	452
E-def.....	14	12	41.0 ±2.4			785	1431		34.0 ±3.0	297	401
Control....	10	14	39.4 ±1.4			965	1700		35.1 ±1.9		
E-def.....	6	14	45.2 ±0.9			846	1419		32.9 ±2.1		
Control....	15	18	33.7 ±1.5	838	1895	1763	2037	21.7 ±2.5	48.2 ±3.0	347	444
E-def.....	12	18	35.1 ±2.0	398	1261	1010	1793	20.7 ±2.5	41.3 ±2.2	326	426
Control....	34	21	38.7 ±2.0	670	2059	1350	1958	22.0 ±2.1	45.8 ±2.5	355	454
E-def.....	13	21	36.7 ±2.3	561	1253	1067	1528	28.5 ±2.1	44.0 ±2.4	348	416
Control....	20	35	27.0 ±1.6	1322	1591	1697	1842	57.4 ±4.0	63.4 ±2.6	395	451
E-def.....	10	35	29.5 ±1.3	909	1403	1146	1644	45.5 ±2.2	56.1 ±2.2	355	422
Control....	9	42	21.0 ±1.7	1580	1820	1707	1878	69.0 ±2.9	70.7 ±2.3		
E-def.....	16	42	20.1 ±1.5	976	1191	1237	1563	61.4 ±2.5	63.4 ±2.0	370	426
Control....	26	28	26.0	1018	1412	1399	1712	48.9	59.4	410	458
E-excess...	22	28	26.9	1084	1466	1450	1735	54.7	61.2	386	455

* Exper. refers to muscles previously subjected to denervation and control to contralateral non-denervated muscles.

† Expressed as per cent of that found in contralateral control.

TABLE 2

A summary of the average values for gastrocnemius muscles of guinea pigs on control, E-deficient and E-excess diets

CONDITION	NO. OF ANIMALS	TIME AFTER LESION	PER CENT WEIGHT LOSS†	TENSION PER GRAM MUSCLE WHEN ACTIVATED THROUGH				RELATIVE STRENGTH OF DENERVATED MUSCLE† WHEN ACTIVATED THROUGH	
				Nerve		Muscle			
				Exper.*	Control	Exper.*	Control	Nerve	Muscle
		days							
Controls.....	17	35	20.8	1007	1643	1237	1834	47.6	54.5
E-excess.....	9	35	24.6	1040	1631	1439	1845	48.2	59.0
E-def.....	9	35	6.2	524	406	737	582	145.2	127.0
Controls	10	14	31.8			669	1778		25.7
E-def.....	6	14	35.5			378	1257		19.4

* Exper. refers to muscles previously subjected to denervation and control to contralateral non-denervated muscle.

† Expressed as per cent of that in contralateral control.

days after denervation by nerve section showed slightly greater losses of strength and weight than was found in similarly treated muscles of control animals on adequate diets (table 2). However, the relatively short duration of the experiment prohibited the development of more than a mild degree of dystrophy in these animals. A study of regeneration in guinea pigs on diets deficient in vitamin E showed that muscles, 35 days after the crushing of their nerves, were appreciably stronger than their unoperated contralateral controls (table 2). This was true for the responses elicited by nerve stimulation as well as by direct activation. Histologic examination showed the presence of dystrophic lesions in both the denervated and control muscles but quantitative estimations of their severity were difficult. The fact that the unoperated contralateral control muscles gave very weak responses to direct and motor nerve stimulation indicated the presence of a severe state of dystrophy in this group of animals. Thus, it would appear as if the effects of a marked E-deficiency were more pronounced on normally innervated muscles than on muscles previously subjected to denervation. It should be noted that Pappenheimer (7) found that nerve section protected the muscles of young suckling rats from the usual effects of E deprivation. Our knowledge concerning the rôle of vitamin E in muscle physiology and the factors that influence the requirements of tissue for the vitamin must be more complete before such phenomena can be satisfactorily explained.

The experiments on different levels of vitamin E intakes show that neuromuscular regneration is little affected by the quantity of vitamin in the diet. If vitamin E is necessary for neuromuscular regeneration, the requirements are so small as to be within the amounts present in an "E-deficient diet" which readily permits the occurrence of lesions characteristic of E-deficiency. These findings on experimental animals suggest the futility of employing large doses of vitamin E in order to promote recovery of muscle and nerve from lesions resulting from causes other than a deficiency of vitamin E itself.

SUMMARY

A comparative study has been made of neuromuscular atrophy and regeneration in the gastrocnemius muscles and tibial nerves of guinea pigs and albino rats on different levels of vitamin E intakes.

The studies included determinations of muscle strength as measured by the response to stimuli applied directly to muscle and to its motor nerve, the degree of atrophy and creatine concentration at various times after unilateral denervation. The muscle and nerve of the contralateral limb served as a control.

The extent and velocity of neuromuscular regeneration in the rat was not significantly affected by the amount of vitamin E in the diet. The intakes ranged from that in E-deficient diets to that in adequate diets supplemented with several times the usual intakes of vitamin E.

Excess intakes of vitamin E exerted no appreciable effect upon the course of neuromuscular regeneration in the guinea pig. The gastrocnemius muscles of E-deficient guinea pigs undergoing regeneration following a unilateral crushing

of the tibial nerve were found to be functionally superior to their contralateral unoperated controls in the tests made 35 days after operation.

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PARATHYROID FUNCTION IN THE HYPOPHYSECTOMIZED RAT

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The production of gross and cellular enlargement of the parathyroid glands of a variety of animals by anterior hypophyseal extracts has led to the assumption that the hypophysis exercises a parathyrotropic function (1). Insufficient evidence has been adduced that these extracts produce any hyperparathyroidism. Some degree of rise in blood calcium level has been reported following their administration (1, 2) but this effect has not been observed by all investigators (3, 4). Furthermore, a fall in serum inorganic phosphate, a characteristic parathyroid hormone effect (5), has not been observed (2, 4, 6).

Although parathyroid atrophy has been found in hypophysectomized animals (1), evidence has not been produced that these glands are functionally insufficient. The serum calcium of hypophysectomized mammals is not significantly depressed (1, 6) nor is the serum inorganic phosphate raised (7). The fall in serum calcium of the hypophysectomized amphibian, Xenopus laevis, has not been shown to be mediated through the parathyroids. Ovariectomy also causes hypocalcemia in this species (8).

Some degree of functional impairment of the hypophysectomized animal's parathyroids might conceivably be present but not be manifest under ordinary experimental conditions. Partial parathyroidectomy may produce slight or no change in the serum calcium and phosphate of animals on a normal diet. The present experiment was designed to test the parathyroid function of hypophysectomized rats subjected to the stress of a low calcium diet. At the same time the susceptibility of hypophysectomized rats to parathyroid insufficiency was determined on account of the possibility that hypophyseal insufficiency might modify in some manner the usual response to parathyroid deprivation (cf. the effect of hypophysectomy on the diabetes of pancreatic ablation).

EXPERIMENTAL METHODS. Male albino rats of ages 60–70 days were used. Hypophysectomy was done by the usual parapharyngeal approach. The completeness of operation was checked at autopsy by making serial sections of any tissue remaining in the pituitary fossa and by body weight curves and the size of the adrenals and testes. Animals failing to satisfy all the criteria were discarded. Parathyroidectomy was done under binocular magnification with removal of as little thyroid as necessary. Occasional animals failing to show the characteristic profound change in blood chemistry were proven to have parathyroid remnants by serial section of the remaining thyroid tissue at autopsy. The double operation (both parathyroidectomy and hypophysectomy) was done on one group of rats at the same time. All animals, both operated and controls, were kept in wire-bottom cages maintained at a temperature of 28–32°C. throughout the duration of the experiment. A recovery period of one to three days after

operation was allowed on the normal diet. The times in the table refer to the period on the experimental diet. The animals were killed at intervals from one to four weeks by anesthetizing with ether and bleeding from the abdominal aorta. The blood was collected directly into centrifuge tubes. Serum calcium was determined on 1.0 ml. serum by the method of Clark and Collip. Serum inorganic phosphate was determined on 0.5 ml. serum by the method of Fiske

TABLE 1

Influence of low calcium diet on the serum calcium and inorganic phosphate of hypophysectomized animals and various controls at weekly intervals

DIET	TIME	NO. RATS	SERUM CALCIUM (MGM.%)			SERUM INORGANIC PHOSPHATE (MGM.%)		
			Lowest	Mean	Highest	Lowest	Mean	Highest
Non-operated controls								
	wks.							
Normal	4	5	10.6	*10.9*	11.6	5.5	*7.0*	8.0
Low calcium	1	4		*10.5*		7.3	*8.0*	8.6
Low calcium	2	5	10.5	*10.8*	11.2	5.3	*7.6*	8.5
Low calcium	3	11	9.9	*10.4*	10.8	6.5	*8.0*	9.7
Low calcium	4	13	7.7	*9.9*	11.6	6.0	*7.0*	9.4
Low calcium	1–4	33		*10.3*			*7.5*	
Parathyroidectomized								
Low calcium	1–2	6	5.2	*6.0*	7.7	12.0	*12.9*	14.8
Low calcium	3	6	5.0	*6.1*	7.3	12.5	*13.1*	13.5
Low calcium	4	6	4.5	*5.4*	6.4	13.5	*16.9*	19.0
Low calcium	1–4	18		*5.8*			*14.3*	
Hypophysectomized								
Normal	4	4	11.0	*11.2*	11.2	4.7	*5.3*	5.6
Low calcium	1	8	9.3	*10.6*	11.0	4.6	*6.8*	8.0
Low calcium	2	9	9.9	*10.6*	11.4	4.1	*5.0*	6.1
Low calcium	3	9	9.7	*10.3*	11.0	5.0	*5.4*	6.1
Low calcium	4	17	9.5	*10.5*	11.6	3.4	*4.7*	6.7
Low calcium	1–4	43		*10.5*			*5.3*	
Double-operated								
Low calcium	1–2	8	4.9	*6.3*	7.1	14.0	*14.9*	15.4

and Subbarow. Individual analyses were made on each rat in most instances. When insufficient serum was obtained for this, analysis was occasionally done on pooled samples. Only occasionally was it not possible to obtain both calcium and phosphate values on the same animal.

The experimental low calcium diet is one which causes marked parathyroid enlargement in rats of this age (9): Alcohol extracted beef fibrin,[1] 20 per cent;

[1] Washed, ground and dried beef fibrin was generously supplied by Dr. J. D. Porsche, Chemical Research Department, Armour & Co., Chicago. It was extracted by refluxing with 95 per cent ethyl alcohol on the steam bath and filtered while hot to remove any vitamin D.

modified Wesson salts,[2] 1.2; KH_2PO_4, 1.8; carotene in oil, 0.1; Wesson oil, 1.9; rice bran extract,[3] 5.0; cane sugar up to 100.0 per cent. This diet contains approximately 0.05 per cent calcium and 0.45 per cent phosphorus almost all of which is inorganic. The normal control diet has the same composition with the addition of 2.5 per cent $CaCO_3$. It maintains normal parathyroid size in rats of this age (9).

RESULTS. The table shows that the serum calcium of the *intact* rats is only slightly lower after 4 weeks on the low calcium diet than that of the controls and that there is no significant difference in the serum phosphates. In marked contrast to this, the *parathyroidectomized* rat has a serum calcium little more than half the normal and a serum phosphate about twice normal. This alteration has taken place before the end of the first week although it tends to become a little more marked in 4 weeks. In spite of this chemical evidence of severe hypoparathyroidism, these rats seldom had manifest tetany.

The *hypophysectomized* rats on the normal diet had a normal serum calcium and a significantly *lower* serum phosphate than the intact animals. We have previously observed this fall in serum phosphate in starved intact animals. It may be a non-specific effect of inanition. It is opposite to the change characteristic of hypoparathyroidism. The low calcium diet produced a very slight fall in the serum calcium of the hypophysectomized rats and no significant difference in serum phosphate after four weeks. The hypophysectomized rats behaved exactly like the intact rats, therefore, in response to the low calcium diet.

Finally, the effect of simultaneous *hypophysectomy and parathyroidectomy* is shown in the last group in the table. The animals withstood the double operation well for the first few days but mortality in this group was high from 5 to 10 days after operation. It was not found practicable to keep them under these experimental conditions for more than two weeks. Within this period of time they had a marked drop in serum calcium and an equally marked rise in serum phosphate. Their reaction was thus indistinguishable from that of the group simply parathyroidectomized.

The average serum protein concentration of 5 hypophysectomized rats kept 4 weeks on the low calcium diet was 5.25 per cent as estimated by the falling drop method. This is a small but significant fall compared to an average of 6.44 per cent in 10 normal rats of the same age on a stock diet. The maintenance of a normal serum calcium is therefore not dependent on a rise in serum protein concentration in the hypophysectomized animals.

DISCUSSION. The functional adequacy of the parathyroid glands of the hypophysectomized rat is well attested by their ability to maintain the serum calcium and inorganic phosphate concentrations within normal limits under the stress of a low calcium diet. No difference has been found between rats hypophysectomized for four weeks and non-operated controls in this respect. At the same time the hypophysectomized rat has been shown to be highly susceptible to para-

[2] Modified to omit calcium and phosphorus salts.

[3] Galen "B", containing vitamins B_1, B_2, B_6, nicotinic acid, pantothenic acid, filtrate factor (The Galen Co., Inc., Berkeley, Calif.).

thyroid deprivation. Since it is known that the parathyroids of intact rats on a low calcium diet of this sort are greatly enlarged, it may be inferred that the parathyroids of the hypophysectomized rat possess a considerable functional reserve.

These results cast a serious doubt on the existence of a direct physiological regulation of the parathyroid glands by the hypophysis analogous to the control exercised over the gonads. Other explanations may be invoked to account for most of the phenomena previously interpreted as evidence of such a function. The atrophy of the parathyroid glands in hypophysectomized animals, for example, is part of a generalized atrophy of organs in these animals (10). Baker (11) found no cytological disturbances in the atrophic parathyroids of the hypophysectomized monkey. It has been suggested by Houssay and Sammartino (12) that this atrophy was due to a generalized nutritional disturbance since it occurred also in their pancreatectomized dogs. It may be significant that our hypophysectomized rats have shown a progressive fall in serum phosphate. A reduction of serum phosphate by restriction of dietary phosphorus is accompanied by atrophy of the parathyroids in rats (13).

Conversely, it may be doubted that the parathyroid enlargement following the injection of hypophyseal extracts is necessarily the direct result of a specific hormonal stimulus. The parathyroid size is remarkably sensitive to dietary alterations and probably to other metabolic factors. It may be significant that very substantial increases in serum inorganic phosphate have been reported after the injection of some hypophyseal extracts (6, 14). The parathyroid enlargement consequent upon renal failure with phosphate retention is a familiar phenomenon and the hyperphosphatemia produced by dietary means may be accompanied by parathyroid enlargement even in the absence of hypocalcemia (13). It would be pertinent also to question the effect of an increase in general metabolism on parathyroid size.

The facts up to this point might be explained on the assumption that hypophyseal secretion increases the demand on the parathyroids. It is not so apparent how a rise in serum calcium following the injection of hypophyseal extracts could be explained on this assumption. However, this reaction has not been observed uniformly and the lack of simultaneous serum protein determinations restricts the interpretation of those results. The efforts made to prove that the reaction was mediated through the parathyroids (1) cannot be regarded as conclusive.

SUMMARY

Evidence has been presented that the function of the parathyroid glands is unimpaired in the young mature male rat deprived of its hypophysis. The hypophysectomized animal maintained its serum calcium and inorganic phosphate concentrations within normal limits as well as the non-operated control even when subjected to the stress of a low calcium diet. In contrast, the hypophysectomized-parathyroidectomized animal under the same conditions suffered a profound fall in serum calcium and rise in serum phosphate, quite like the

parathyroidectomized control. The significance of the parathyroid atrophy following hypophysectomy and of the influence of anterior hypophyseal extracts on parathyroid size and serum calcium, reported by other investigators, has been discussed.

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THE REVERSIBILITY OF THE INHIBITION OF RAT BRAIN AND KIDNEY METABOLISM BY COLD[1]

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A new interest in the physiological effects of hypothermia on homeothermic animals has developed as a consequence of the work of Smith and Fay (cf. 1), which has shown that body temperatures of the order of 24°C. can be maintained in human beings for some time without ill effects. That further reduction in body temperature would be unwise is suggested by various observations. Available data indicate that non-hibernating mammals usually do not survive if their body temperature falls to a level of 15° to 20°C. Critical levels of hypothermia have been reported as about 13 to 16° for the rat (2); 20° for the rabbit (3); 16° for the cat (4); 14° (one case) for the monkey (5) and 22° for the dog (6). While the lethal level of hypothermia is thus fairly clear for the animals mentioned, the cause of death in the non-hibernating mammal on acute exposure to cold is not clear. It has been noted by several investigators that in the animal dying of hypothermia the heart continues to beat for some time after cessation of respiration (4, 5, 6). However, it is possible that the respiratory failure is consequent upon impaired circulation. It has been shown that a critical fall in arterial pressure occurs in the dog at about 22°C. (7). Anoxia so established would lead to failure of medullary and other nervous centers (cf. Gerard, 8). It is also possible that death is due to direct and irreversible effects of cold on the brain rather than to failure of the supply mechanisms such as respiration and circulation. The experiments reported here were designed to throw some light on this latter possibility. Besides the work on excised brain, some measurements of respiration were also made on kidney cortex slices to see how this organ, which appears to have the highest metabolic rate of the several organs of the rat (9), would be affected by profound cooling.

METHODS. Five adult rats of the Slonaker-Wistar strain were used. They were killed by decapitation. Measurements of oxygen consumption and of anaerobic glycolysis were made by manometric methods, which have been described in previous communications (10). Procedures peculiar to the problem in hand were these. Oxygen consumption or anaerobic glycolysis was measured for an hour in a thermostat at 37.7° ± 0.01°C. The respirometers were then transferred to another thermostat maintained at 0.2° ± 0.02°C. Fifteen minutes were allowed for thermoequilibration, then readings were taken every fifteen minutes, as before, at this new and lower temperature level. In four experiments the respirometers were transferred back to the first thermostat (at 37.7°C.) after one hour at 0.2°C. (75 min. if the thermoequilibration period be

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included). Again fifteen minutes were allowed for thermoequilibration and readings were taken for the ensuing hour. In one experiment, using cerebral cortex slices from one animal, respirometers were kept in the cold bath at 0.2°C. for periods of one, three, five, seven and twenty-four hours before being restored to the warm bath. Points to note are that the rates of cooling and rewarming were kept constant and that the duration of exposure to cold was constant with the exception just described.

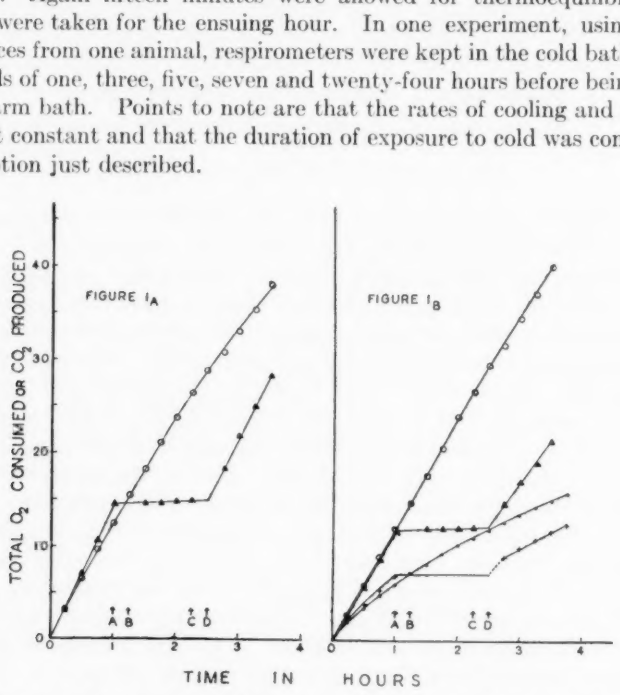

Fig. 1-A. Graph showing the total oxygen consumed, in cubic millimeters, N. P. T., per milligram dry weight by kidney cortex slices as a function of time and temperature. ○ = control. Total oxygen consumed. Temperature constant at 37.7°C. ▲ = experimental. Total oxygen consumed. Temperature changed as indicated by arrows. *A:* experimental vessels transferred to 0.2°C. *B:* end of thermoequilibration period at 0.2°C. *C:* experimental vessels returned to 37.7°C. *D:* end of thermoequilibration period at 37.7°C.

Fig. 1-B. Graph showing total oxygen consumed and total carbon dioxide produced (anaerobic) in cubic millimeters, N. P. T., per milligram dry weight, by rat cerebral cortex slices as a function of time and temperature. ○ = control. Total oxygen consumed. Temperature constant at 37.7°C. ▲ = experimental. Total oxygen consumed. Temperature changed as indicated by arrows. ● = control. Anaerobic glycolysis. × = experimental. Anaerobic glycolysis. A, B, C, D, same as in figure 1-A.

When results are given as rates (differential curves) they are expressed in cu. mm., N.P.T., of oxygen (respiration) or of carbon dioxide (anaerobic glycolysis) consumed or produced per mgm. dry weight per hour (Q_{O_2} and $Q_G^{N_2}$ respectively). When given in terms of total gas consumed or produced as a function of time (integral curves) the units on the Y-axes are cu. mm. gas, N.P.T., per mgm. dry weight.

RESULTS. 1. *Kidney cortex.* It is shown in figure 1-A that the respiration of rat kidney cortex slices drops to a very low level at 0.2°C. This level is fully established at the end of the fifteen minute equilibration period and does not

change, within the limits of error of our measurements, for the ensuing hour. When the tissue is returned to the bath at 37.7°C. there is complete recovery of the capacity for oxygen consumption. In fact, the respiration of the rewarmed tissue is at a somewhat greater rate than that of the control which was not refrigerated. This is perhaps attributable to a decreased catabolism of respiratory catalysts as well as of other substrate at the low temperature.

2. *Brain cortex.* a. *Oxygen consumption.* It is shown in figure 1-B that there is very complete recovery of the respiratory capacity of rat cerebral cortex slices after a period of one hour at 0.2°C. (with 15 min. periods for equilibration). Again, the rate of oxygen consumption is quite constant at the low temperature. However, when the time of exposure to 0.2°C. is lengthened there is progressive diminution in the recovery of oxidative capacity. Thus, after periods of one, three, five, seven and twenty-four hours at 0.2°C., the values of Q_{O_2} obtained during one subsequent hour at 37.7° were 8.50; 7.75; 7.35; 7.22 and 4.43 respectively. The control value (first hour at 37.7°) was 9.07 (each of these values is the mean of determinations run in duplicate). In every case the values of Q_{O_2} were constant at the levels given for the one hour after rewarming during which readings were made.

b. *Anaerobic glycolysis.* It is shown in figure 1-B that recovery of the capacity for anaerobic glycolysis after one hour at 0.2° can be regarded as complete. Insofar as these data are concerned, there is no evidence that anaerobic glycolysis is more liable to damage by cold than respiration. It is interesting that the same "preservation" effect obtained with kidney cortex respiration is obtained here. Thus the rate of anaerobic glycolysis after rewarming, which is during the third hour of the run, is about that of the control during the second hour at 37.7° and definitely greater than the rate of the control during the third hour at 37.7°.

Discussion. As far as we know, neither the respiration and anaerobic glycolysis of mammalian cerebral cortex slices nor the respiration of mammalian kidney cortex slices have previously been measured during and after exposure to a temperature as low as 0.2°C. There are data on the respiration of minced rat brain over the temperature range 25° to 45°C. (11) and on rat cerebral cortex slices over the range 5° to 42°C. (12). The only other mammalian cell species for which we have found similar observations in the literature is sperm. Chang and Walton (13) have shown that if the temperature of a suspension of ram sperm be lowered gradually (5°C. stages with an interval of two hours at each stage), the cells can be cooled to 1°C. and kept at this level for twenty-four hours without loss in respiratory capacity. Using other criteria of recovery than regained respiratory capacity it has been shown that a certain percentage of mammalian sperm can survive exposure to much lower temperatures (14, 15). In view of the findings of Chang and Walton (13), it is possible that the reduction of Q_{O_2} observed in our twenty-four hour run would not have occurred if the cooling had been more gradual.

However, it is shown by our experiments that rat cerebral cortex slices can recover their initial capacity for oxygen consumption and for anaerobic glycolysis after an hour of exposure to a temperature of 0.2°C. even when the cooling and

rewarming are so rapid that they are complete in fifteen minutes or less. This temperature is far below the lethal level for the intact rat, which is about 13° to 16°C. (2). If respiration of the brain as a function of temperature follows the same course *in vivo* and *in vitro*, and if recovery of a normal level of oxygen consumption implies recovery of other functions of the brain, then it follows that death of the intact rat in acute severe hypothermia is not attributable to direct and irreversible action of cold on the brain but rather to failure of the supply mechanisms, circulation and respiration. Irreversible damage in the intact animal is probably due to anoxia of the central nervous system resulting from this failure. In contrast to this picture of failing oxygen and nutrient supply to the brain *in vivo* at low body temperatures stands the continued adequacy of the supply of oxygen and glucose to the tissue slices in the respirometer vessels. Thus it is that the intact rat dies at 13° to 16°C., while the cerebral cortex slice in the respirometer does not show impaired oxidative capacity even after an hour at 0.2°C.

SUMMARY

1. The rate of oxygen consumption of rat kidney cortex slices, measured for an hour at 37.7°C., then for an hour at 0.2°C., was restored to the initial level on rewarming to 37.7°. The periods of cooling and of rewarming did not exceed fifteen minutes each.

2. The rates of oxygen consumption and of anaerobic glycolysis of rat cerebral cortex slices, determined under the conditions described above, also showed full recovery on rewarming.

3. When the duration of the period of exposure of brain slices to 0.2°C. was increased to three, five, seven and twenty-four hours, there was progressive decrease in the rate of oxygen consumption regained on rewarming.

4. The significance of these observations in relation to the death of non-hibernating mammals from acute hypothermia was discussed.

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THE EXCRETION OF SULFANILAMIDE AND ACETYLSULFANILAMIDE BY THE KIDNEY OF THE RABBIT[1,2]

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Sulfanilamide is eliminated from the mammalian body almost entirely through the urinary excretory system. It appears in the urine of most mammals in two forms. One is the "free" or uncombined form and the other is the "conjugated" or combined form. Combined sulfanilamide has been shown to be acetylsulfanilamide (1). Stewart and collaborators (2) have shown that in the rabbit this acetylation takes place in the liver.

At the present time there are few data available concerning the mechanism by which the kidney excretes sulfanilamide. Marshall and co-workers (3) have studied the mode of excretion of this compound in dogs by comparing its renal clearance with the simultaneous clearance of creatinine. Their studies suggest that sulfanilamide is filtered through the glomerulus and partially reabsorbed through the tubule. During the course of a study on the excretion of urea and urea derivatives, Nichols and Herrin (4) obtained sulfanilamide clearances which by similar interpretation would lead to essentially the same conclusions as those of Marshall and co-workers. Stewart and associates (5) using a modified clearance technique on humans, and Marshall and co-workers (3) using dogs, found that acetylsulfanilamide has a considerably higher clearance than does the free drug.

The present study is concerned with the mechanism by which the kidney of the rabbit excretes sulfanilamide and acetylsulfanilamide and the factors which influence the excretion of these compounds. The method used involves comparing the renal clearance of these drugs with the simultaneous clearance of one or more compounds the mechanism of excretion of which has been established.

METHODS. Five full grown male rabbits were used in this study. The animals were fasted for at least eight hours previous to each experiment, but they were allowed to drink water as desired.

The unanesthetised animal was tied on his back on the animal board and a number 18 or 20 plain rubber catheter was inserted in the bladder. It remained in the bladder throughout the experiment. The drugs used were either sulfanilamide or acetylsulfanilamide plus inulin, creatinine and diodrast.[3] They were dissolved in 0.9 per cent saline so that the concentration of the compounds was about 400 mgm./100 cc. each of sulfanilamide or acetylsulfanilamide, creatinine

[1] This study was made in partial fulfilment of the requirements for the degree of Doctor of Philosophy by T. A. Loomis.

[2] This investigation was aided by a grant from the Junior Board of the Buffalo General Hospital.

[3] The diodrast used in this work was kindly furnished by the Winthrop Chemical Company.

and inulin, and about 75 mgm./100 cc. of diodrast. This solution was administered to the rabbit by gravity through a hypodermic needle inserted in a marginal ear vein. The rate of administration of the fluid was controlled by a screw clamp on the rubber tubing below a glass drip indicator. In order to obtain rapidly a desired concentration of the drugs in the blood, the infusion was allowed to run into the vein at a rate of about 5 cc. per minute for ten minutes. Following this a rate of approximately 1 cc. per minute was maintained throughout the remainder of the experiment.

At the end of the rapid infusion period, the bladder was washed with 20 cc. of warm normal saline by means of a syringe attached to the end of the catheter. In exactly 30 minutes the bladder was washed again and the washings were added to the urine formed during the 30-minute period. This was repeated at the end of a second 30-minute period so that two clearance periods were obtained in each experiment. A blood sample was taken at the midpoint of each period by intracardiac puncture and was immediately centrifuged. Potassium oxalate was used in a few of the experiments, but heparin was the anticoagulant usually employed.

All chemical analyses were carried out on a common protein-free plasma filtrate. The filtrate was prepared according to the second zinc sulfate-sodium hydroxide method of Somogyi (6). The drug levels were determined in the plasma filtrates and the urine samples by the same methods. Sulfanilamide and acetylsulfanilamide were determined by the method of Bratton and Marshall (7), inulin by the method described by Hubbard and Loomis (8), creatinine according to Langley's method (9), and diodrast by Alpert's method (10). It was found that recoveries of added inulin, creatinine, sulfanilamide and acetylsulfanilamide from plasma were identical whether the filtrate was made with Somogyi's agents or with the precipitating agents originally suggested by the authors of the various methods. It was also shown by appropriate experiments that the presence of all of these drugs in a common plasma filtrate in the concentration used in the experiments did not interfere with the determination of any one of them.

The clearance of the drugs was calculated according to the formula UV/P, where U and P equal the respective urine and plasma concentrations of the drug, and V equals the urine flow in cubic centimeters per minute.

Results. The results of 14 periods in which the clearances of sulfanilamide, creatinine, inulin and diodrast were determined simultaneously are summarized in table 1. In ten of these periods sulfanilamide was injected and in four periods acetylsulfanilamide was injected and the determinations were based on measurable amounts of free sulfanilamide formed in the animal. Table 1 also summarizes the results of 28 simultaneous clearance periods of acetylsulfanilamide, creatinine, inulin and diodrast. Nineteen of these periods were obtained after administering preformed acetylsulfanilamide and 9 after it was formed by the animal from sulfanilamide. The mean clearances of acetylsulfanilamide and creatinine are almost identical (4.9 and 4.8 cc. per min. respectively). Although the mean clearance of inulin (3.9 cc. per min.) is lower than that of

either of the two former compounds, statistical analysis of the series reveals that the mean clearance of inulin is not significantly different from the mean clearance of creatinine or acetylsulfanilamide.

The clearances of both sulfanilamide and acetylsulfanilamide are independent of the urine flow which varied in the experiments from 0.2 to 1.2 cc. per minute. The clearances of these two compounds are also independent of their plasma concentrations, and it was shown that the rate of excretion (urine concentration times urine flow) of both of these drugs increased when their plasma concentration is increased. During the course of the study the plasma concentration range of sulfanilamide was 1 to 25 mgm. per cent and of acetylsulfanilamide was 0.1 to 6.0 mgm. per cent.

Discussion. Marshall and associates (3) have shown that sulfanilamide is filterable through membranes which are impermeable to the plasma proteins and this has been confirmed in this laboratory. However, there is some evidence that about 20 per cent of the sulfanilamide present in normal plasma is bound to the plasma proteins (11, 12). This would leave only 80 per cent of the drug free to pass through the glomerular membrane. The observed differences between the clearances of inulin or creatinine and sulfanilamide (the ratio of the inulin or creatinine clearance to the sulfanilamide clearance is approximately 3 to 1) is much too great to be explained by the presence of a non-filterable fraction of the magnitude reported. Since the renal clearance of inulin is generally accepted as a measure of the glomerular filtration rate in all mammals in which this has been studied, and since Kaplan and Smith (13) found that the creatinine and inulin clearances are identical in the rabbit, the observed ratio of the inulin or creatinine to the sulfanilamide clearances suggests that sulfanilamide passes through the glomerular membrane and is partially reabsorbed through the tubule from the glomerular filtrate.

During the course of this study it was found that the rabbits were not only able to acetylate sulfanilamide but they were also capable of hydrolysing acetylsulfanilamide after its administration. They were not all able to hydrolyse it to the same extent, consequently some plasma analyses showed only traces of the free drug. Four clearance periods were obtained when it was formed following administration of the conjugated drug, and the mean clearance of sulfanilamide in these four periods (2.3 cc. per min.) was about the same as the mean clearance obtained (1.8 cc. per min.) when the free drug was given directly.

In order to interpret the data which was obtained on acetylsulfanilamide it was necessary to devise an experiment to determine whether this compound will pass through the glomerular membrane. The following experiment was done: acetylsulfanilamide was added to fresh rabbit plasma and the solution was filtered under reduced pressure through a sheet of number 600 cellophane. Repeated determinations showed that the filtrate was protein-free and contained acetylsulfanilamide in the same concentration as the plasma. These results which were similar to the results of Marshall et al. (3) upon sulfanilamide suggest that acetylsulfanilamide in blood plasma can be filtered through the glomerular membrane. The ratio of the mean clearance of acetylsulfanilamide to that of

creatinine and inulin was found to be 1.02 and 1.24, respectively. Therefore, if the creatinine clearance is accepted as a measure of the glomerular filtration rate, the clearance of acetylsulfanilamide is also a measure of that rate. Since evidence indicates that acetylsulfanilamide will pass through the glomerular membrane, these data may be interpreted as meaning that this compound is excreted solely by glomerular filtration. In addition the clearance of acetylsulfanilamide was found to be independent of the plasma concentration of the drug, and its rate of excretion increased with increases in the plasma concentration. These characteristics are true for any compound which is excreted solely by glomerular filtration.

In a manner similar to that shown to be true for sulfanilamide, the mean clearance of acetylsulfanilamide was found to be essentially the same whether it was determined on the preformed compound (19 periods, average clearance is

TABLE 1

Comparison of simultaneous renal clearance periods

PERIODS	COMP. USED	MEAN AND P.E. OF MEAN	MEAN DIFF.* / P.E. OF DIFF.	RATIO OF MEANS
14	S	1.8 ± 0.1	S I = 8.6	S/I = 0.40
	C	6.0 ± 0.3	S C = 11.8	S/C = 0.30
	D	26.1 ± 1.5	C I = 3.0	C/I = 1.31
	I	4.6 ± 0.3		
28	AS	4.9 ± 0.3	AS C = 0.3	AS/C = 1.02
	C	4.8 ± 0.2	AS I = 2.6	AS/I = 1.24
	D	25.2 ± 1.3	C I = 2.4	C/I = 1.23
	I	3.9 ± 0.2		

* In this study the following rule was used: If the mean difference between two columns of figures is 4 or more times the probable error of the difference, this difference is not due to chance but the two means are significantly different.

I = inulin; C = creatinine; D = diodrast; S = sulfanilamide; AS = acetylsulfanilamide.

4.6 cc. per min.) or on the compound formed in the animal from sulfanilamide (9 periods, average clearance is 4.9 cc. per min.).

The diodrast clearance may be used as a reasonably accurate measure of the plasma flow to the kidneys if the plasma concentration of the compound is maintained below a level at which its clearance becomes self depressed (14, 15, 16, 17). The plasma levels were below 8.4 mgm./100 cc. in all experiments in this series. The diodrast clearances at the highest levels attained are in the same range as the clearances obtained when the plasma levels of this compound were 1.0 to 2.0 mgm./100 cc. Thus it appears that the diodrast clearances at the highest plasma levels used in this study were not significantly depressed. The mean clearance of diodrast was found to be approximately 25 cc. per minute. The glomerular filtration rate as determined by the creatinine, inulin or acetylsulfanilamide clearance was approximately 4 to 5 cc. per minute. This means that about one-fifth of the volume of blood plasma delivered to the rabbit's kidney passes through the glomerular membrane and forms the glomerular

filtrate, a figure which is comparable with the proportion of plasma filtered in the dog's kidney as reported by Van Slyke and associates (18).

An attempt was made to determine whether the presence in the plasma of any one of the compounds used in these experiments either increased or decreased the clearance of any of the others. During the course of this study eight clearance periods were obtained in which either two or three of the usual four compounds were given to the animal. In these periods it was found that the clearances were not significantly different from those obtained when all four of the compounds were administered. Thus in these experiments it does not appear that any one of the compounds, in the concentrations which were used, materially influences the clearances of the others.

SUMMARY AND CONCLUSION

1. In rabbits the clearance of sulfanilamide was found to be 30 to 40 per cent of the simultaneously determined clearance of creatinine and inulin respectively. This together with evidence based upon ultrafiltration experiments which indicate that sulfanilamide will pass through the glomerular membrane is interpreted as meaning that sulfanilamide is filtered through the glomerular membrane and partially reabsorbed from the glomerular filtrate.

2. The clearances of acetylsulfanilamide and creatinine were found to be almost identical. Furthermore, the clearance of neither of these compounds differed significantly from the clearance of inulin. This together with evidence based on filtration experiments suggesting that acetylsulfanilamide is free to pass through the glomerular membrane is interpreted as meaning that acetylsulfanilamide is excreted by the rabbit solely by glomerular filtration.

3. The clearances of sulfanilamide and acetylsulfanilamide in this study were found to be independent of their plasma concentration as well as the urine flow.

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THE INJURY CURRENT IN THE ELECTROCARDIOGRAM[1]

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Injury to the surface of the mammalian heart produces deviation of the S-T segment of the electrocardiogram, the direction of which depends upon the location of the injury. Injury to the right ventricle causes a depression, and injury to the left ventricle an elevation of the S-T segment with reference to the diastolic base-line. The extent of the injury determines the degree of elevation or depression (1).

Consideration of the probable explanation of this phenomenon led to a series of experiments based on the following assumptions.

1. Injury to the surface of a ventricle leads to the production of an injury potential.

2. The direction in which the injury potential is oriented in relation to the leads, and consequently its effect upon the leads, depends upon the orientation in space of the injured region.

3. Since the injured surface is in the negative field of the injury potential and the rest of the heart in its positive field, the anatomical relationships would necessitate that the current of injury would cause a downward deflection of the string when the injury is on the left ventricle and an upward deflection of the string when the injury is on the right ventricle.

4. During systole the source from which the injury current arises becomes depolarized, the injury current disappears and the string should return to the isoelectric level. Whether the source of the injury current is visualized as the result of a relative negativity of injured as compared to normal tissue or as the result of dipoles standing across the "membrane" of demarkation between injured and uninjured tissue, the electrical changes as they affect remote leads would be the same.

The habit of considering the diastolic position of the string as the normal base line or the isopotential level has prevented investigation of this problem, as has also the difficulty of producing an injury to the heart *in situ* while the electrocardiogram is being recorded. The following experiments were designed to circumvent this difficulty.

METHOD. A string galvanometer was employed for these experiments, because it will maintain a constant deflection in the presence of a constant potential. Instruments with condenser-coupled amplifiers obscure the phenomenon because of the tendency of the beam to return to a center position despite

[1] Aided by a grant from the Fluid Research Funds, Yale University School of Medicine.

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the presence of constant potentials. Thirteen dogs were employed, prepared as previously described (1).

Experiments of two types were performed. In the first, a small pledget of cotton wool wrung out in Ringer's solution was placed at the apex of the left ventricle or over the conus of the right ventricle. A short segment of fine-bore rubber tube was led into the pledget, and the chest wound closed around it. The animal was placed so that the pledget was on the most dependent portion of the heart. Recording was begun of an electrocardiogram from lead II or III, and continued while 0.5 to 2.0 cc. of M/5 KCl was injected rapidly into the pledget. The regions studied are best represented in those leads.

In the second type of experiment a small pledget (1–2 cm. in diameter), moistened in M/5 KCl, was placed on the apex of the left ventricle or on the conus of the right ventricle. Over it were placed in succession a silver-silver chloride disk electrode (1.0 cm. in diameter), a somewhat larger sheet of dry blotting paper or rubber dam, and finally a second silver-silver chloride electrode. The electrodes were connected by fine insulated wires to a switch, by means of which the insulation between the two plates could, in effect, be removed or applied at will. This was then done while the electrocardiogram was being recorded from lead II or III. The same type of experiment was performed on four animals in which an irreversible injury was produced by a burn at the left apex, and on five in which an infarct was produced by multiple ligation of vessels leading to the apex of the left ventricle.

Results. Figure 1 shows the results of application of M/5 KCl to the surface of the left ventricle (A) and of the right ventricle (B). It can be seen that the actual deviation is that of the base-line, and that the S-T take-off remains at the original iso-electric line. Such fluctuations as are seen in the height of the S-T take-off are interpreted as resulting from respiratory and other changes in the position of the heart. Injury to the right ventricle causes an upward deviation of the base-line, while injury to the left ventricle is followed by downward deviation of the base-line.

Figure 1, C and D, illustrates the results of the second series of experiments, in which insulation over an injured region was temporarily nullified by establishing the circuit between the plates inside and outside of the insulation. Short-circuiting the insulation over a KCl injury of the right ventricle produced an upward deviation of the base-line, and a "depressed" S-T take-off which actually remained at the level of the former base-line (fig. 1C). Restitution of insulation was followed by return of the base-line to its former level and restoration of the normal S-T take-off.

Nullification of insulation over a KCl injury of the left ventricle produced a downward deviation of the diastolic base-line, and an "elevated" S-T take-off which actually remained at the iso-electric level (fig. 1D).

Experiments with burned areas were performed only on the left ventricle. The results were the same as those obtained with KCl on the left ventricle (fig. 2D). Circumvention of the insulation produced a downward deviation of the base-line and an "elevated" S-T take-off, which in fact remained at the iso-electric level. Restoration of the insulation was followed by return of the de-

pressed base-line to its original level and the disappearance of the deviation in the S-T take-off.

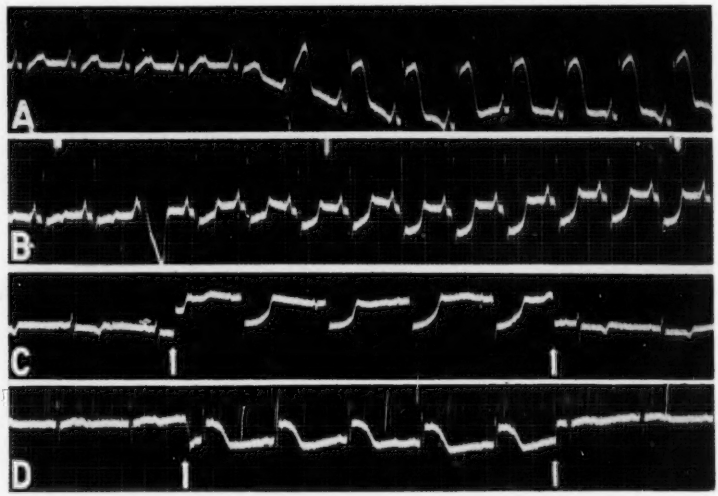

Fig. 1. A, B. July 31, 1942. Dog, 7.5 kgm. Dial anesthesia. A. Animal on left side; 1.0 cc. M/5 KCl injected on to left ventricle. Lead III. B. Animal on right side; 2.0 cc. M/5 KCl injected on to right ventricle. Lead III. C, D. August 6, 1942. Dog, 7.0 kgm. Dial anesthesia. C. Pledget soaked in M/5 KCl on anterior surface of right ventricle. Insulation was short-circuited between arrows. Lead III. D. Pledget soaked in M/5 KCl on left ventricle near apex. Insulation was short-circuited between arrows. Lead III.

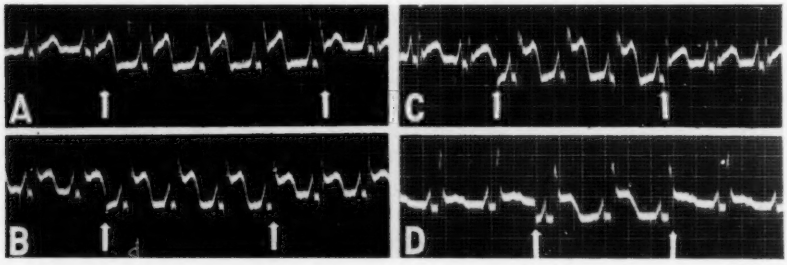

Fig. 2. A, B, C. November 3, 1942. Dog 9.0 kgm. Dial anesthesia. Infarct on posterior surface of left ventricle near apex. A. Small infarct totally insulated by small insulation size of plates (2.0 cm. in dia.). Between arrows the insulation was short-circuited. B. Larger infarct not totally covered by insulation the same size as short-circuiting plates. Between arrows the insulation was short-circuited. C. Large infarct totally covered by insulation much greater in area than the short-circuiting plates. Between arrows the insulation was short-circuited. D. September 9, 1942. Dog, 8.0 kgm. Dial anesthesia. Insulation of burned area on left apex 1.5 cm. in diameter. Between arrows the insulation was short-circuited. All records are lead III.

Figure 2 A illustrates the result of short-circuiting and restoring insulation over an area of infarction at the left apex produced by ligation of coronary arteries. It can be seen that an injury potential is present in myocardial infarction and is

identical with that produced by injury with KCl or burning. The S-T take-off is "elevated" only because the diastolic base-line is depressed. Actually, the S-T take-off remains at the original isopotential level.

It was noteworthy that, whenever the insulation was short-circuited, or restored, during the S-T interval, no displacement of the string occurred. If the insulation was short-circuited at this time, the base-line was displaced only at the end of T. We have records of 10 experiments, of which figure 2A is an illustration. Injury was produced by infarction (4 cases), burning (3 cases) and potassium (3 cases). In no instance was there a change in the tracing coincident with the shunting in or out of the insulation during the S-T period. No current is being generated at the site of injury and whether the insulation shields this region or not can make no difference. Figure 1C is not an exception for the reason that recovery had already begun.

Whether a positive phase of the action potential exists, whether injury is necessary to show it and what the conditions are that make it most manifest, are questions on which the present experiments throw no certain light. Ideas on the subject are confused. Some work indicates that an injured region of the ventricle becomes quite as positive during activity as it is negative during diastole (2). Other experiments performed in a similar fashion indicate that the injured muscle is very much more negative at rest than it is positive during activity (3, fig. 1). Still other work has it that the region of injury (partial potassium depolarization) is negative during rest and iso-electric during activity (4, p. 43).

If the injured region comes into the positive field of action potential during systole and is in the negative field during diastole, it is difficult to see why current in the ordinary leads would not reverse completely from systole to diastole instead of being essentially monophasic. Except for the superimposed QRS deflections the string seems to vary from the iso-electric level in one direction only in any single experiment.

There are apparent exceptions to this rule, as indicated by the following. A large quantity of KCl was infused on to a cotton pledget, laid at the apex of the left ventricle, while the electrocardiogram was being recorded (10–20 cc. instead of 0.5–1.0 cc.). The S-T take-off was then often above the original isopotential line instead of at the same level. Possible explanations are: 1, that injury was sufficient to produce a positive injury action potential; 2, that the potassium solution, spilling generously over the intrathoracic organs, induced injury currents that were not quenched by the systolic depolarization of the heart muscle; 3, that partial injury was produced in other regions of the heart beyond those more completely injured in which depolarization was incomplete but which failed to respond to excitation.

A second set of circumstances gave similar appearances (fig. 2C). A large area of the left ventricle was injured by infarction or by application of KCl. The injured area was shielded by a large insulating layer, so that the expected S-T "elevation" was slight or absent. The plates used to short-circuit the insulation were smaller than insulation or the injured area. When these plates were connected, the S-T take-off was above what it had been before. This may be taken as evidence for a positive injury action potential or may more simply be due to

the fact that when the plates were connected the injury current spread to the leads from a different and more concentrated source than would have been the case if the plate electrode had been co-extensive with the injury.

When a large injured area is partly covered by a sheet of insulating material of relatively small area, the injury current is not wholly shielded from the leads and the S-T segment is "elevated." If two plates just covering the two sides of the insulation are connected and the insulation thus eliminated, the S-T segment remains at exactly the same level and has the same contour as it had before. The injury current can flow more freely now during diastole and the diastolic parts of the record are lowered (see fig. 2B). The fact that variations in the resistance to current flow make no difference in the systolic parts of the curve is compatible with the view that no current flows from the injured region to the leads during systole.

Discussion. These experiments establish the fact that injury to the surface of the heart produces a persistent displacement of the diastolic base-line of the electrocardiogram. This displacement is upward when the right ventricle is injured and downward when the left ventricle is injured. Results of previous experiments in which potentials of known polarity were impressed across the heart between the right and left ventricles have demonstrated that, when an electrode at the surface of the right ventricle is made negative with respect to an electrode at the surface of the left ventricle, there is an upward displacement of the beam, while negativity at the left ventricular electrode causes a downward displacement of the beam (5). It can therefore be inferred that the persistent diastolic deviation of the string in the present experiments is due to the establishment of a persistent potential so oriented that the injured regions are in the negative field and the uninjured parts are in the positive field. Whether this injury current flows between closely placed dipoles at the boundary of injured and non-injured tissue or between the surfaces of the right and left ventricles, the deviation of the string recorded in the lead studied would be expected to be the same.

During systole the injury current disappears, as is shown by the return of the string to the original base-line, and by the absence of displacement of the string when insulation is short-circuited during the S-T interval. At the end of systole the injury current appears. This disappearance and reappearance of the current of injury describes a monophasic curve upon which are superimposed the QRST deflections which result from the activity of normal cardiac muscle.

The S-T take-off therefore occurs at the iso-electric level, indicating at this point the abolition or equalization of potential differences in the portions of the heart represented in the lead studied.

"Elevation" of the S-T segment means in reality, therefore, the depression of the diastolic base-line by a current of injury resulting from injury to the left ventricle.

"Depression" of the S-T segment denotes the elevation of the diastolic base-line by a current of injury resulting from injury to the right ventricle. The degree of displacement is an index of the degree or extent of injury.

Whether the agent producing the injury be KCl, a burn or a myocardial in-

farct, the nature and direction of the injury current are the same. Whether the injury is extremely superficial, as with KCl, or whether it is deep, as with thermocoagulation or an infarct, is likewise without influence on the resulting deviation.

Other factors than the presence of an injury current may give rise to a deviation of the S-T take-off. Examples may be found in electrocardiograms of bundle-branch block, tachycardia, digitalis intoxication and ventricular ectopic beats. In these circumstances terminology may be more clear if we speak of this situation as a superimposition or fusion of QRS and T, rather than deviation of the S-T segment, preserving the latter term for the above described function of the injury current. Differentiation into these two categories should not be difficult except where both phenomena occur together.

These observations permit a more complete statement of a standpoint which has been taken in earlier papers of this series. It was hypothesized that potassium suppressed electrical activity on the surfaces where the material was spread, and that the resulting monophasic deflection was due to the activity of the uninjured portion of the heart. It is now evident that the potassium or other injury suppresses the phasic activity of the injured tissue and produces an injury current. The monophasic deflection is due to suppression of this current, by the activity of tissue which remains capable of phasic response. It is possible that the injury current is quenched only as the whole heart becomes depolarized, but there is no evidence in the present experiments contrary to the idea that the injury current is produced by dipoles standing across the demarkation "membrane" which separates injured from uninjured tissue and that the process of excitation depolarizes this "membrane" locally.

CONCLUSIONS

1. Injury to the right ventricle causes an upward displacement of the diastolic base-line in the three standard leads of the electrocardiogram. Injury to the left ventricle produces a downward displacement of the diastolic base-line.

2. During systole there is a return of the string to the isopotential level which constitutes the S-T segment.

3. In reality, therefore, elevated S-T segments must be interpreted as due to an injury potential resulting from left ventricular damage, which produces in the electrocardiogram a downward displacement of the diastolic base-line.

4. Depressed S-T segments must be interpreted as due to an injury potential resulting from right ventricular damage, which produces in the electrocardiogram an upward displacement of the diastolic base-line.

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THE EFFECT OF POSITIVE AND NEGATIVE INTRA-THORACIC PRESSURE ON PERIPHERAL VENOUS PRESSURE IN MAN[1]

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In an earlier paper (Holt, 1941) it was shown that in the anesthetized dog in the supine position peripheral venous pressure is not a function of right auricular pressure when right auricular pressure is decreased. Evidence was presented that this is due to the fact that when right auricular pressure is decreased the veins become partially collapsed just before entering the chest, increase the resistance to the flow of blood along the veins, and maintain peripheral venous pressure constant when right auricular pressure is decreased.

Recently Ryder, Molle and Ferris (1941) have stated that in most normal human subjects peripheral venous pressure is a function of tissue pressure and is independent of right auricular pressure. The experiments reported in this paper were performed in order to determine if peripheral venous pressure is a function of right auricular pressure in man.

METHODS AND RESULTS. Venous pressure was measured in the antecubital vein by a modification of the direct method of Moritz and Tabora (1910) using 0.85 per cent sodium chloride solution in the manometer system. In the supine position the venous pressure was referred to the level of the middle of the chest as zero (von Recklinghausen, 1906). In the sitting position the venous pressure was referred to the lower end of the sternum as zero. As eleven or more venous pressure determinations were made in each experiment, a slow continuous injection of saline was made between each venous pressure determination in order to prevent clotting.

The subject breathed through a mouthpiece from a breathing chamber in which the pressure could be varied from twenty centimeters of water above to twenty centimeters below atmospheric pressure. The breathing chamber had a volume of approximately twenty-five liters. A continuous flow of fifty liters or more of air passed through the chamber each minute in order to keep the air in the chamber about the same composition as that of room air. The pressure in the chamber varied around one centimeter of water with each respiration. The subject breathed from the chamber continuously, and whenever the pressure in the chamber was changed, one to three minutes was allowed before the venous pressure reading was made.

Peripheral venous pressure was measured in eight subjects in the supine position, lying on a flat table, with the arm abducted to approximately forty-five degrees and held well below heart level. The subjects breathed from the cham-

[1] A preliminary report of this work was given at the meeting of the American Federation for Clinical Research in Minneapolis, 1942.

ber in which the pressure was varied from fourteen centimeters of water above atmospheric to fourteen centimeters below. Figure 1 shows the results of a typical experiment. As the chamber pressure was increased, peripheral venous pressure increased and as the chamber pressure was decreased peripheral venous pressure decreased. There was a linear relationship between peripheral venous pressure and chamber pressure as shown in figure 1. It appears that when air under the above negative pressures was breathed there was no collapse of the veins and that peripheral venous pressure was a function of right auricular pressure.

In three subjects the experiment was performed as described above with the exceptions that a maximum inspiratory effort lasting thirty seconds was made against a mercury manometer. In these experiments the intra-thoracic pressure was decreased below atmospheric by forty centimeters of water or more. The peripheral venous pressure fell seven centimeters or less during the first fifteen or twenty seconds and then started to rise. When these large negative intra-

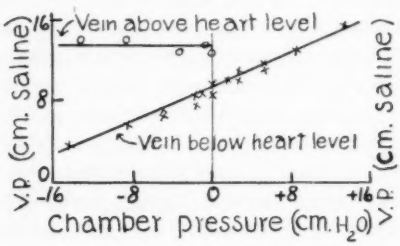

Fig. 1

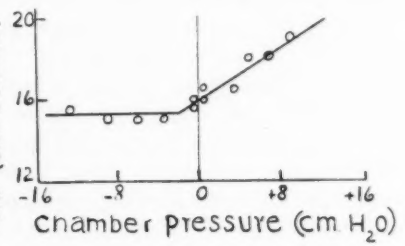

Fig. 2

Fig. 1. The effect of changing the breathing chamber pressure on peripheral venous pressure in the supine subject. *V.P.*, venous pressure. +, above atmospheric pressure. −, below atmospheric pressure.

Fig. 2. Same as figure 1 but with the subject in the sitting position.

thoracic pressures were developed, it appears that the peripheral venous pressure fell to a point where the veins became partially collapsed, and beyond this point peripheral venous pressure was independent of right auricular pressure.

In three subjects the experiment was performed as described in the first group of experiments with the exception that the antecubital vein was elevated to a point well above heart level. The peripheral venous pressure, referred to the heart as zero, was increased when the arm was placed in this position. This was due to the fact that the peripheral veins became partially collapsed (Lyons, Kennedy and Burwell, 1938; Holt, 1940). When the subject breathed air that was under a negative pressure the peripheral venous pressure remained constant (fig. 1). Thus when the arm was elevated well above heart level it appears that the peripheral venous pressure was not a function of right auricular pressure because the veins became partially collapsed in the periphery.

It was thought that with the subject in the sitting position the column of blood extending from the upper end of the thoracic cage down to the right auricle

would exert a negative pressure on the veins entering the upper end of the thoracic cage and tend to collapse them. In order to determine if there was a partial collapse of the veins entering the upper end of the chest, venous pressure was measured in the antecubital vein of three subjects in the sitting position with the arm abducted to approximately forty-five degrees and resting on a board. The subject breathed from the chamber as in the above experiments. When air under positive pressure was breathed the peripheral venous pressure was increased, and when air under negative pressure was breathed the peripheral venous pressure remained constant (fig. 2). Thus it appears that when air under a negative pressure was breathed, there was a collapse of the veins between the point where the venous pressure was measured and the right auricle, and that peripheral venous pressure was not a function of right auricular pressure. Since, in the sitting position, the blood ran upward from the point in the vein where the pressure was measured to the upper level of the thoracic cage it would appear that there was not a collapse of the veins in the periphery but that the collapse occurred at the point where the veins enter the upper end of the chest.

Discussion. Since right auricular pressure was not measured in these experiments, there was the possibility that a change in intra-thoracic pressure was not associated with a change in right auricular pressure. However, since it has been shown in the dog that right auricular pressure is a linear function of intra-thoracic pressure (Holt, 1941), and since in the supine human with the arm held below heart level a rise in intra-thoracic pressure is associated with a rise in peripheral venous pressure and a decrease in intra-thoracic pressure is associated with a fall in peripheral venous pressure, it would appear that intra-thoracic pressure changes are transmitted to the right auricle.

Although it has been shown in the dog that peripheral venous pressure is not a function of right auricular pressure when right auricular pressure is decreased, the data presented here indicate that in man peripheral venous pressure is a function of right auricular pressure when the subject is supine and the arm is below heart level. This difference would appear to be due to the fact that in the supine dog right auricular pressure is atmospheric or slightly sub-atmospheric, while in man in the supine position the pressure in the right auricle is several centimeters of saline above atmospheric (Richards et al., 1942). As a result a small decrease in right auricular pressure in the dog causes the veins to become partially collapsed just before entering the chest, thus making peripheral venous pressure independent of right auricular pressure. However, in man right auricular pressure may be lowered considerably before the pressure in the veins outside the chest becomes low enough to cause them to become partially collapsed, and until that point is reached peripheral venous pressure is a function of right auricular pressure.

When the arm is elevated several centimeters above heart level in the supine position the veins become partially collapsed peripherally because the pressure inside of them is less than that outside, and as a result changes in right auricular pressure do not cause a change in the peripheral venous pressure. With the subject in the sitting position the veins appear to become partially collapsed

at the point where they enter the upper end of the thorax and peripheral venous pressure is not a function of right auricular pressure.

There is the possibility in those experiments where peripheral venous pressure remained constant when the intra-thoracic pressure was decreased that as a result of the decreased intra-thoracic pressure cardiac output was increased and the rate of flow of blood along the veins of the arm was increased, thus keeping the peripheral venous pressure constant. This is unlikely because in the supine position with the arm below heart level, the peripheral venous pressure decreased when the intra-thoracic pressure was decreased.

SUMMARY

Venous pressure was determined in the antecubital vein by a modification of the direct method of Moritz and Tabora in eight normal subjects who breathed from a chamber in which the pressure was varied from fourteen centimeters of water above to fourteen centimeters below atmospheric. In the supine subject, with the arm held well below heart level, the peripheral venous pressure decreased when air under negative pressure was breathed, and increased when air under positive pressure was breathed. When the arm was held well above heart level, in the supine subject, the peripheral venous pressure remained constant when intra-thoracic pressure was decreased. In the sitting position the peripheral venous pressure remained constant when the intra-thoracic pressure was decreased.

In normal man, in the supine position with the arm well below heart level and abducted to forty-five degrees, peripheral venous pressure is a function of right auricular pressure.

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ON THE EXCRETION OF IODINE IN THE SALIVA[1]

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The excretion of iodine in the saliva after the oral or parenteral administration of iodine compounds has received comparatively little attention to date. In 1929 Lipschitz (1) showed that in dogs, following the intravenous injection of sodium iodide in doses ranging from 20 to 50 mgm. per kilogram, the parotid saliva attained a concentration of iodine 5 to 7 times that of the blood. Even greater concentrations were observed in the gastric secretion. Barkan and Leistner (2) reported simultaneously that when an organic iodine compound "Jodtropon" was fed to a dog, so-called "organic" iodine failed to appear in either salivary or gastric secretions In 1930, Lipschitz (3) showed that in dogs only minimal amounts of iodine appeared in the saliva or gastric juice after the intravenous administration of large doses of thyroxine He assumed that the small amounts of iodine secreted in these body fluids represented inorganic iodine gradually released from the circulating thyroxine.

In 1931, Schneider and Widmann (4) found the concentration of iodine in human saliva to vary from 0 to 362 micrograms per 100 ml. Scheffer (5) also found the excretion of iodine in the saliva to be marked at times. Bruger, Hinton and Lough (6), however, found the iodine content of normal human saliva to vary between 3.5 and 24.0 micrograms per 100 ml. with a mean of 10.2 ± 5.1. Undoubtedly, some of the high figures for human salivary iodine heretofore published were obtained from subjects either ingesting or exposed to abnormal amounts of iodine. In a recent report, Flexner, Bruger and Member (7) demonstrated that in man, after the intravenous injection of sodium iodide, inorganic iodine is selectively concentrated and secreted by the salivary and gastric glands.

Thienes and Hockett (8) studied the rate of intestinal absorption of potassium iodide in man after the subcutaneous injection of an extract of the posterior lobe of the pituitary. The time of appearance of measurable quantities of iodine in the saliva was used to determine the rate of absorption of iodide. Their results indicated that the appearance of iodine in the saliva was delayed 40 minutes after the injection of the pituitary extract. Heath and Fullerton (9), utilizing a spot test to detect iodine in the saliva, found that after the oral administration of 0.25 gram of potassium iodide to 8 normal subjects, iodine appeared in the saliva within 10 to 15 minutes. In 21 patients, all but two showed no detectable salivary iodine after 20 hours. They found the intestinal absorption of iodide to be delayed in a wide variety of clinical conditions including patients who had achlorhydria or who suffered from some disease process likely to condition a disturbance of the absorptive mechanism.

[1] Aided by a grant from the Thyroid Fund of the Thyroid Clinic, Departments of Medicine and Surgery, New York Post-Graduate Hospital.

MATERIAL AND METHODS. The present studies were carried out on 16 normal subjects (usual hospital personnel) and on one patient with essential hypertension. Persons assigned to surgery were uniformly excluded because of probable contact with iodine. Unless indicated, the experiments were done in the morning after breakfast. Wide-mouthed bottles used to collect the saliva, after thorough cleaning, were rinsed with iodine-free distilled water. Each subject chewed a small amount of paraffin to insure an adequate flow of saliva; from 1 to 5 ml. were collected at stated intervals.

In the oral experiments, the iodine compound was administered in a gelatin capsule with particular precautions taken to insure that the outside of the capsule was not contaminated with iodine. Approximately 200 ml. of water was

TABLE 1

The iodine content of the saliva (mgm. per 100 ml.) of seven normal subjects following the ingestion of potassium iodide in amounts ranging from 50 to 500 mgm. (38 to 380 mgm. of iodine)

SPECIMEN	GROUP I 50 MGM. POTASSIUM IODIDE (38 MGM. IODINE)							GROUP II 100 MGM. POTASSIUM IODIDE (76 MGM. IODINE)		GROUP III 500 MGM. POTASSIUM IODIDE (380 MGM. IODINE)	
	D. O.	M. B.	E. McL.	S. M.	M. G.*	M. G.	M. S.	E. McL.	E. McD.	M. G.	M. B.
hours											
0	0.013	0.013	0.015	0.019	0.005	0.044	0.025	0.045	0.008	0.008	0.012
½	1.692	1.226		2.609	2.553						
1	2.547	2.428	2.143	2.861	3.517						
1½	2.646	2.365	1.634	2.366	2.961	2.392	2.168	3.247	9.182	9.770	13.225
2	3.111	1.932	2.345	2.642	3.391						
2½	3.335	2.249	2.446	2.725	2.133						
3		1.737	2.057	2.396	1.846	1.800	1.217	2.462	3.428	7.319	9.845
6		1.912	1.458	2.153	1.400	1.991	0.794	2.120	3.416	8.085	8.055
12		0.741	1.025	1.370	1.000	1.468	0.718	0.990	1.805	6.100	3.037
24		0.177	0.177	0.574	0.155	0.314	0.143	0.388	0.969	1.475	1.489
48		0.028	0.056	0.048	0.045	0.083	0.034	0.063	0.196	0.379	0.314
72		0.011	0.021	0.016		0.018	0.009	0.024	0.040	0.030	0.068
96									0.025	0.008	0.020

* Fourteen hours post-prandial.

imbibed to wash down the capsule. The mouth was then rinsed with a dilute solution of alcohol. Potassium iodide, iodine dissolved in poppy seed oil,[2] ethyl diiodobrassidate[3] and sodium tetraiodophenolphthalein were thus administered. In the parenteral experiments, sodium iodide in aqueous solution was injected intravenously. The method of Trevorrow and Fashena (10, 11) was used throughout.

RESULTS. *Experiments with iodides.* Table 1 shows the salivary iodines of 7 normal subjects before and after the oral administration of 50 to 500 mgm. of potassium iodide (38 to 380 mgm. of iodine). The maximal concentration of iodine in the saliva occurred in 1 to 2½ hours after the ingestion of the iodide;

[2] Lipiodol (Fougera).
[3] Lipoiodine (Ciba).

following 50 mgm. of potassium iodide it reached 700 times that of the control level and after 100 to 500 mgm. 1100 to 1200 times the initial salivary iodine. Abnormal amounts were still detected in the saliva 48 hours following the ingestion of the iodide but after 72 or 96 hours normal values were usually obtained.

In one patient with essential hypertension, whole blood and salivary iodines were done before and at frequent intervals (total period of 48 hrs.) after the ingestion of 50 mgm. of potassium iodide (38 mgm. of iodine). Maximal concentrations of iodine in both body fluids were noted at the 2-hour period (0.126 mgm. per 100 ml. of whole blood and 2.974 mgm. per 100 ml. of saliva) but augmented values persisted in the saliva for a longer time. The salivary/blood iodine ratio in the control period was 6 and reached a maximum of 28, 12 hours after the ingestion of the iodide.

TABLE 2

The iodine content of the saliva (mgm. per 100 ml.) of 8 normal subjects following the intravenous administration of sodium iodide in amounts ranging from 25 to 100 mgm. (22.4 to 89.6 mgm. of iodine)

SPECIMEN	GROUP I 25 MGM. SODIUM IODIDE (22.4 MGM. IODINE)		GROUP II 50 MGM. SODIUM IODIDE (44.8 MGM. IODINE)			GROUP III 100 MGM. SODIUM IODIDE (89.6 MGM. IODINE)		
	L. W.*	E. F.*	L. K.*	E. B.*	E. R.*	S. B.	W. K.	S. E.*
minutes								
0	(0.011)	(0.008)	(0.012)	(0.007)	(0.009)	(0.009)	(0.012)	(0.011)
5	3.80	4.00	8.40	3.15	6.70	14.30	6.70	14.30
10	2.90	2.70	5.67	2.94	3.80	11.10	8.20	
15	2.90	2.30	7.77	2.73	4.40	10.10	7.80	11.60
30	2.10	2.10	9.03	2.52	4.00	12.20	8.80	12.60
45	1.90	2.10	7.77	2.10	3.60	12.00	7.60	10.30
60	1.70	1.70	9.24	1.89	3.80	8.20	5.70	8.60
90	1.50	1.50	9.24	1.68	2.90	10.10	7.80	8.60
120	1.40	1.30	6.30	1.89	2.50	7.40	4.80	7.00

* Fourteen hours post-prandial.

Table 2 demonstrates the salivary iodines of 8 normal subjects before and after the intravenous administration of 25 to 100 mgm. of sodium iodide (22.4 to 89.6 mgm. of iodine). The appearance of high concentrations of iodine in the saliva as early as 5 minutes after the injection was noted. In fact, the greatest concentration occurred at that time, then gradually tapered off but iodine was still present in the saliva in appreciable amounts two hours after the injection. Apparently, iodine was secreted in greater concentration in the saliva following intravenous injection than after the oral administration of an equivalent amount of iodide.

Experiments with iodine dissolved in poppy seed oil. In six studies on four normal subjects, salivary iodines were determined before and at frequent intervals (total period of 168 hrs.) after the ingestion of a capsule containing 200 mgm. of iodine dissolved in poppy seed oil. The increased values for salivary iodine were not unlike those obtained with equivalent amounts of iodine as

potassium iodide. Concentrations of iodine in the saliva as high as 5.0 or 6.0 mgm. per 100 ml. were reached occasionally. In 3 experiments, no food was ingested for 14 hours prior to the investigation but this factor appeared to exert little influence on the concentration of iodine in the saliva.

Experiments with ethyl diiodobrassidate. In one normal subject, 123 mgm. of iodine as ethyl diiodobrassidate was ingested on three separate occasions (adequate intervals were allowed between experiments for the salivary iodine to return to normal levels). The highest value for salivary iodine obtained was 5.675 mgm. per 100 ml. In two of the three experiments, however, the maximal concentration did not occur until 5 or 6 hours after the ingestion of this compound.

Experiments with sodium tetraiodophenolphthalein. Two normal subjects ingested a capsule containing 100 mgm. of sodium tetraiodophenolphthalein (55.2 mgm. of iodine). Iodine in appreciable amounts failed to appear in the saliva for as long as 72 hours after the ingestion of the dye.

COMMENT. The discrepancy between the present results and those of Heath and Fullerton (9) on the duration of the increased salivary iodine after the ingestion of potassium iodide may be ascribed to the more accurate analytical procedure used in this investigation. It will be recalled that Heath and Fullerton used a spot test (the addition of ferric chloride and starch to saliva and noting the appearance of a blue color at the end of 20 min.) and observed that in almost all their subjects, iodine could not be detected in the saliva 20 hours after the ingestion of potassium iodide. Our results show that more than normal quantities of iodine may still be secreted in this fluid as long as 48 hours and occasionally 72 hours after the oral administration of an equivalent amount of iodide. This preferential secretion of iodine by the salivary glands sets up an internal circulation ("inneren Kreislaufs") (1)) since the iodine of swallowed saliva is absorbed from the intestinal tract and again finds its way into the saliva by way of the blood stream.

The salivary glands are impermeable to iodine administered as sodium tetraiodophenolphthalein. Assuming that these glands cannot secrete iodine in organic combination because of the increased molecular size of such compounds (2, 3), it would appear that tetraiodophenolphthalein fails to undergo cleavage in the body at least to the extent of releasing iodine in elemental form or in simple inorganic combination. In one patient with obstruction of the common bile duct, the saliva contained only 0.02 mgm. of iodine per 100 ml. whereas the whole blood attained a value of 2.10 mgm. per 100 ml. four days after the administration of sodium tetraiodophenolphthalein for gall-bladder visualization. Of added interest may be cited the observations in a patient with a broncho-biliary fistula. Two hours after the administration of this dye, the whole blood showed a concentration of 12.78 mgm. per 100 ml. and the saliva (mixed with sputum and bile) contained 19.22 mgm. per 100 ml. Obviously, the liver in sharp contrast to the salivary glands is able to secrete iodine compounds of appreciable molecular size.

On the other hand, some organic compounds of iodine may undergo fairly

rapid cleavage in the body. One may infer that the high salivary iodines noted after the ingestion of ethyl diiodobrassidate represents the release of iodine from the iodine-fatty acid complex, presumably by the action of the intestinal lipases. The delayed maximal concentration of salivary iodine after the ingestion of ethyl diiodobrassidate would favor this concept.

CONCLUSIONS

1. Marked increases in the concentration of iodine in the saliva were noted in man after the oral administration of potassium iodide, iodine dissolved in poppy seed oil and ethyl diiodobrassidate.

2. The maximal concentration of salivary iodine occurs in approximately 1 to 2½ hours after the ingestion of potassium iodide or iodine dissolved in poppy seed oil but may be delayed for 5 or 6 hours after the oral administration of ethyl diiodobrassidate. More than normal amounts of iodine in the saliva may still be detected as long as 72 hours after the ingestion of these substances.

3. After the intravenous administration of sodium iodide, the maximal concentration of iodine in the saliva occurs within 5 minutes.

4. The iodine content of the saliva fails to increase after the oral administration of sodium tetraiodophenolphthalein.

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ARTERIAL AND CENTRAL VENOUS PRESSURE CHANGES FOLLOWING COMPLETE TRANSECTION OF THE SPINAL CORD (C8–T1)

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It is commonly accepted that arterioles and perhaps capillaries are maintained in a partially constricted state by vasotonic impulses which emanate from supra-segmental centers. The removal of this central influence and the consequent diminution of peripheral resistance and augmentation of the capacity of the peripheral vascular bed are employed to explain the initial state of hypotension which develops abruptly after division of the spinal cord at the 8th cervical level. The question has recently been raised whether the importance of normal vasotonic control is not greatly over-emphasized (1). From the evidence submitted by the latter investigators, it would appear more exact to attribute the hypotonic response in the spinal animal to a *passive* post-arteriolar dilatation of capillaries, venules and veins in consequence of the withdrawal of their extra-vascular skeletal muscle support. It is presumed that the capacity of these elastic venous structures is primarily regulated by skeletal muscle tone. In accordance with this interpretation, spinal section should lead to reduced skeletal muscle tone, passive dilatation and engorgement of capillaries, veins and venules, reduced venous return and right atrial pressure, impairment of ventricular filling and finally diminution of the stroke volume and thus a lowering of arterial pressure. It becomes a question then as to whether vascular or extra-vascular factors are the primary precipitants of this hypotensive response.

The following studies were conducted to ascertain the changes in *a*, heart rate; *b*, atrial and effective venous pressures; *c*, arterial pulse pressure; *d*, intrathoracic pressure, and *e*, the form of central arterial pressure pulses which are associated with the development of arterial hypotension in the acute spinal animal.

PROCEDURE. This study was conducted on 12 cats. To ascertain whether the removal of extravascular support by rendering the skeletal musculature flaccid is an important feature in the hypotension which develops in the supine spinal animal it was necessary to maintain essentially normal muscle tone for control observations. Hence, these cats were lightly anesthetized with pentobarbital so as to reduce sensory perception without seriously impairing skeletal muscle tone. The doses employed varied from 25 to 28 mgm. of pentobarbital per kilo of body weight.

Operative procedures entailed preparation for simultaneous optical registra-

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tion of the central arterial (carotid), the atrial and intrapleural pressure curves. Arrangements were made to measure mean carotid pressures from a critically damped mercury manometer at regular intervals. A thin-walled stainless steel sound (bore 2.5 mm.) was employed for transmission of atrial pressure fluctuations to a sensitive optical membrane manometer. It was introduced via the right jugular vein until its tip rested either just inside the right atrium or at the junction of this chamber with the superior vena cava. It was also rendered possible to read atrial pressure fluctuations as transmitted to a saline manometer at desired intervals. Intrapleural pressure changes were transmitted through a trocar fixed in the chest wall to another optical membrane manometer. Heparin was used systemically to prevent clotting in the atrial sound, thus eliminating the bizarre and unreliable atrial pressure curves which occur as a result of partial occlusion of the transmission system.

Atrial pressures fluctuate almost continuously throughout the cardiac cycle. What point on the curve, then, represents the pressure most effective for the regulation of ventricular filling? One group of investigators have recently found that measurement of pressures at the peak of the *a* wave yielded the most consistent results in dogs (2). This method of measurement is unsatisfactory in cats, particularly when tachycardia is present, because the *a* wave is frequently masked or entirely absent. We have adopted the expedient of measuring the pressure prevailing just prior to the sudden decline of the V-wave of the venous pulse. Such a procedure seems advisable since this point is usually easily detected. It also conforms with the concept that the major portion of ventricular filling (rapid inflow phase) is governed by the "effective venous pressure" near the end of the isometric relaxation phase. This latter pressure, as defined by Henderson and Barringer, is the algebraic difference between atrial and intrathoracic pressure just prior to the opening of the A-V valves (3). Since there is a cyclic variation in these pressures coinciding with the respiratory cycles, it seemed advisable to measure atrial pressure only from those pulses which coincided with expiratory apnea and with the peak of inspiration. Since the depth of inspiration also varies from moment to moment, it was decided to utilize for discussion purposes only those atrial pressures measured during expiration. The latter remained relatively constant during control observations.

A comparison of atrial pressure values obtained from precisely measured atrial pressure curves with the rough estimates derived from reading rapid saline manometer excursions convinced us that the latter values are very unsatisfactory. Expiratory values from the saline manometer were consistently too low. Inspiratory values were usually too high, though they occasionally equalled or even fell considerably below pressures measured from optical records. It has been our experience that saline manometer readings of atrial pressure cannot be relied upon to indicate either the direction or magnitude of a trend unless atrial pressure changes are extremely severe. In the latter instance, the accuracy of measurement is still unsatisfactory.

The experimental procedure was as follows: After circulatory equilibrium had been established following all preparatory procedures, control records and pres-

sure readings were taken at five minute intervals for 25–30 minutes. The spinal cord was then transected by a method, devised by one of us, which eliminates the necessity of tissue exposure and laminectomy (4). The transection was always at the 8th cervical level. This procedure required from three to five minutes in different animals. Observations were then continued at regular intervals for one hour. At the end of this period, animals were sacrificed and the extent of the cord lesion determined. Those cords not cleanly severed were preserved and examined histologically at some later date.

RESULTS. (a) *Control period.* Skeletal muscle tone in these animals appeared to be reasonably normal. Attempts to passively flex and extend the extremities were opposed by considerable resistance. Spontaneous movement of the limbs was occasionally observed. In most of the animals, control respiration was eupneic in character. Cats 10 and 11 were hyperpneic and bordering on dyspnea, due it is believed to the incidence of pneumothorax incurred while or after inserting the intrapleural trocar. Hence, control atrial, intrapleural and effective venous pressures in these animals were abnormal and are not included in the data presented in this paper. It is noteworthy that these bizarre respirations did not alter the arterial blood pressure picture significantly with the result that the arterial pressure changes in these animals are included with the others. At this stage of the experiment, the nictitating membranes were partially relaxed, a common observation in cats under light pentobarbital anesthesia.

To conserve space, fairly representative results from only one of our 12 experiments are presented in table 1. The average trend of the fairly uniform events is depicted in graphic form in figure 1. In the latter, the direction, if not always the magnitude, of individual responses is accurately represented. A perspective of the average control values is presented in tabular form below:

MM. OF HG				MM. OF SALINE					
				IAP		IPP		EVP	
H.R.	S.P.	D.P.	M.B.P.	INS.	EXP.	INSP.	EXP.	INSP.	EXP.
190	154	109	129	−30	+32	−81	−26	+51	+58

Arterial pressures were well within the range of normalcy for pentobarbitalized cats. The atrial and effective venous pressures compare favorably with those previously reported as normal for barbitalized dogs (5).

(b) *Spinal transection period.* With this blind spinal puncture technique the operations were completed within three to five minutes. During a portion of the procedure, as in other methods, the spinal cord was mechanically irritated. As a result, arterial, atrial and effective venous pressures were abruptly elevated. Arterial pressures well above 200 mm. of Hg were observed in every animal. The systolic pressure was elevated to a much greater extent than the diastolic pressure, hence pulse pressures were greatly augmented. The heart rate was moderately retarded and on a few occasions momentary cardiac irregularities developed.

(c) *Primary post-operative phase.* This phase is arbitrarily limited to the

period required for arterial blood pressure equilibrium to be regained. It varied from 10–20 minutes in the different animals. During this time, arterial and atrial pressures descended from their artificially elevated levels, the former more

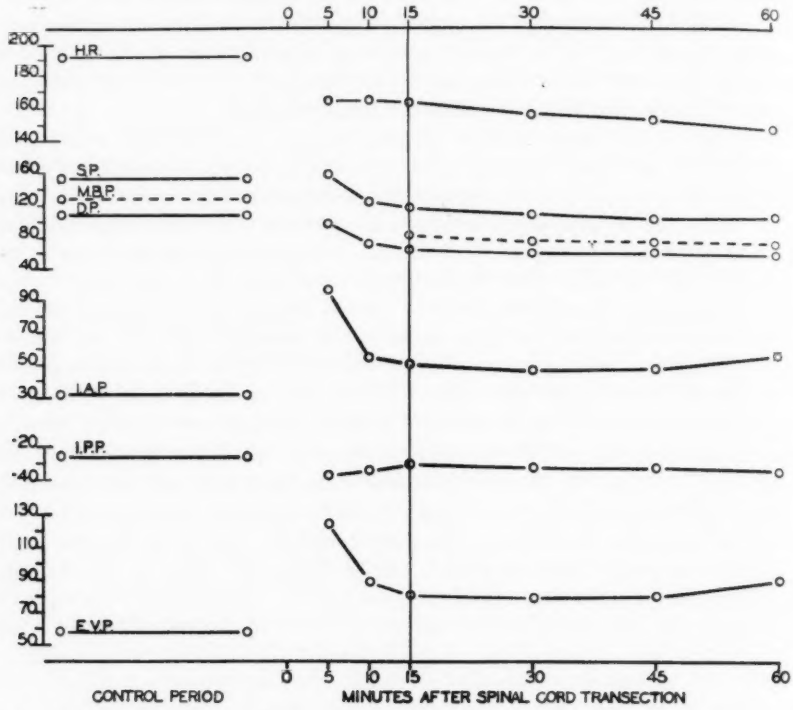

Fig. 1. Average responses of 12 cats whose spinal cord was transected at the 8th cervical level. *H.R.*, heart rate; *S.P.*, systolic pressure; *M.B.P.*, mean blood pressure; *D.P.*, diastolic pressure; *I.A.P.*, intra-atrial pressure; *I.P.P.*, intrapleural pressure, and *E.V.P.*, effective venous pressure.

TABLE 1*

EXPERIMENT NO. 4	CONTROL VALUES	CHANGES AFTER TRANSECTION					
		5′	10′	15′	30′	45′	60′
Heart rate (beats per minute)	153	120	138	135	130		
Systolic pressure (mm. of Hg)	170	188	152	139	130	135	125
Diastolic pressure (mm. of Hg)	120	120	98	92	82	89	85
Pulse pressure (mm. of Hg)	50	68	54	47	48	46	40
Mean blood pressure (mm. of Hg)	134			98	80	94	90
Atrial pressure (mm. of saline)	35	40	30	40	60	70	60
Intrapleural pressure (mm. of saline)	−25	−50	−40	−10	−35	−33	−35
Effective venous pressure (mm. of saline)	60	90	70	50	95	103	95

* A copy of similarly tabulated results for all 12 experiments will be furnished on request.

rapidly than the latter. By the end of the *primary phase* arterial pressures were fairly well stabilized at subcontrol levels. The pulse pressures rapidly approached or assumed preoperative values. The form and slopes of the arterial pressure pulses were likewise unchanged generally. In cats 7 and 12, however, bizarre pulses developed which made the accurate measurement of pulse pressures impossible. As will be mentioned later, these two animals were probably in a state of irreversible circulatory shock.

Atrial pressures descended slowly. By the end of the primary phase, they were again approaching stabilization at supra-control levels. In no instance did they fall significantly below their respective control levels. A similar course was followed by effective venous pressures. Intra-pleural pressures of the expiratory cycles were not appreciably altered by cord transection. The changes in the rate of respiration were neither of significant magnitude nor uniform in direction.

(d) *Secondary post-operative phase.* This constitutes the remainder of the hour of post-operative observation. It varied from 40–50 minutes in duration. This was a period of minor changes with little apparent significance in this problem. Arterial pressures had not begun to approach control values by the end of this period. There are reasons for speculating that arterial pressure recovery was delayed by the use of a non-volatile anesthetic. When similar hypotensive responses were elicited in etherized or decerebrate cats, the degree of recovery was quite striking in many animals within the hour following spinal transection (6).

(e) *Miscellaneous post-operative findings.* Skeletal muscles innervated behind the cord lesion were deprived of their pre-operative tone early in the *primary phase*, prior to the decline of arterial blood pressure. The hind limbs in particular displayed a complete flaccidity. Preoperative tone in skeletal muscles innervated above the lesion was either retained or augmented as the experiments progressed. Respiration, originally costodiaphragmatic, was rendered purely diaphragmatic after cord section. In spite of this, intrapleural pressure relationships were not disturbed. It appears that diaphragmatic movements are alone capable of sustaining adequate pulmonary ventilation in the cat under these conditions. The nictitating membranes were completely relaxed after transection.

Gross and histological examination of the injured cords yielded anatomical evidence that bilateral division of the spinal cord was complete in all save animal 8. The latter appears to have undergone a right hemisection of the cord, even though its functional manifestations were similar to those observed in the other cats.

Discussion. Arterial pressures in these lightly anesthetized supine cats began to decline abruptly after spinal cord transection at the 8th cervical level. Circulatory equilibrium was again re-established at hypotensive levels within 10–20 minutes (*primary phase*). In all save two animals (7 and 12), these subcontrol arterial pressures exceeded 70 mm. of Hg. There was no evidence that these animals suffered the condition of circulatory shock. Their pressure pulse

contours were essentially those observed during the control periods. It seems quite likely that these cats would have survived if the period of observation had been considerably extended. The situation in animals 7 and 12 was complicated by immeasurable hemorrhages which were associated with the operative procedures. In these, arterial pressures dipped to "critical levels." Arterial pressure pulses revealed "high systolic peaks" with little or no diastolic gradients, a type commonly encountered in hemorrhagic and shock-like conditions. It is suspected that these two animals would have become fatalities if the period of observation had been prolonged. Circulatory shock apparently does not occur as a result of spinal cord transection when mechanical trauma and hemorrhage are curtailed to a minimum.

The question arises as to which of the mechanisms involved in the maintenance of normal arterial blood pressure are eliminated or impaired in the animal rendered acutely spinal. It is believed that the relative importance of cardiovascular as compared with extra-vascular mechanisms in sustaining normal pressures in the supine animal becomes evident after the various cardiodynamic events associated with the decline of arterial pressure during the *primary phase* are analyzed on the basis of established fundamental hemodynamic concepts. It is judged, a priori, that the hypotensive response to spinal transection was a manifestation of one or more of the following dynamic events; *1*, a diminution of arterial resistance, *2*, a retardation of heart rate, *3*, an impairment of ventricular stroke volume, and *4*, an increase in the capacity of the system of Windkessel structures.

Under controlled experimental conditions, events *1* and *2* favor a predominant lowering of diastolic pressure and hence an augmentation of pulse pressure. On the other hand, events *3* and *4* favor a diminution of the pulse pressure since systolic pressures are predominantly lowered. Any one of these events acting singly would alter pulse pressures significantly. It appears that several of these events were involved in a reaction in which systolic and diastolic pressures were reduced to approximately the same extent.

Hemodynamically, the major portion of the diastolic pressure decline must be attributed to dynamic changes *1*, *2* or both. Slowing of the heart rate appears to be rather inconsequential in this situation. Despite moderate retardation, heart rates remained markedly tachycardic. The thought occurs that the moderate slowing may have tended to prevent a greater reduction of arterial pressure through the expedient of permitting more adequate ventricular filling at each stroke, thus improving the systolic discharge. It is also noteworthy that equivalent hypotensions developed in two animals in which heart rates were slightly accelerated after spinal transection. This leads to the belief that the major portion of the decline in diastolic pressure is primarily the result of dilatation of peripheral arterioles due to the interruption of vasotonic impulses which normally maintain arterioles in a partially constricted state. This is the usual explanation of the decline in blood pressure in the spinal animal. It is obvious, however, that this event alone cannot explain the equivalent decrease in systolic pressure.

Recent observations by H. Smith and his collaborators might seem to be pertinent in this respect (1). The inference is drawn from their work that the elastic veins and perhaps capillaries are deprived of their normal extravascular skeletal muscle support after transection of the cord and that the capacity of the great venous reservoir is passively augmented. As a result, it is assumed that there is considerable pooling of blood in the peripheral venous system with a consequent reduction of atrial and ventricular filling pressure which must lead to a reduction of systolic discharge. At least a partial answer to this suggestion that a reduced systolic discharge is involved in this systolic pressure decline is obtained from the observations that atrial and effective venous pressures, if altered at all, were slightly elevated during the *primary phase*, while the state of hypotension was developing. Furthermore, the slopes of the ascending limbs of arterial pressure pulses were not reduced as would be anticipated if the ventricle were beating hypodynamically because of inadequate filling. Therefore the atrial pressures and ventricular stroke volumes in these supine animals do not appear to have suffered when the skeletal musculature of the thorax, abdomen and hind extremities were abruptly rendered completely flaccid. It is quite reasonable however to assume that this factor would assume major importance if the animals' position were altered such that it was necessary to counteract the effects of gravity on the venous return.

So far as these supine animals are concerned, there remains by elimination the probability that factor 4 (increased capacity of aorta and large arterial branches) is primarily responsible for the major reduction of systolic pressure. Unfortunately, little is known at present of the physiological characteristics of the Windkessel system. There is evidence, however, that the diameter of the aorta changes markedly in response to reflex and humoral stimulation (7). It is not unreasonable, then, to assume that the muscular elements of the Windkessel system, like the smaller peripheral vessels, are normally subject to vasotonic impulses which maintain the large vessels in a partially constricted state. The capacity of these vessels would thus increase when the regulatory influences were removed by spinal transection. This then may be the mechanism which accounts for the fall in systolic pressure not accounted for by the arteriolar dilatation.

SUMMARY AND CONCLUSIONS

This study concerns the acute arterial hypotension and associated phenomena which occur following transection of the spinal cord (C8) of supine lightly anesthetized cats. When the transection procedures were uncomplicated by severe hemorrhage, pressures did not descend to critical levels. The hypotension developed after the skeletal musculature of the thorax, abdomen and hind limbs, which supposedly support the elastic post-arterial vessels, had been rendered completely flaccid. Apparently, however, the venous return of these supine animals was not impaired by the loss of this extravascular support. The criteria for this concept are based upon the fact that atrial and effective venous pressures were, if anything, slightly elevated, while the heart rates remained

tachycardic. The slopes of the ascending limbs of arterial pressure pulses revealed no changes indicative of an impaired stroke output.

In explaining the equivalent decrease of systolic and diastolic pressures on a hemodynamic basis, it was deduced that reduction of peripheral resistance is a major contributing factor, but that some additional dynamic event must be involved. The analysis suggests that an increase in the capacity of the aorta and its immediate large branches is involved. The acute hypotension following spinal transection may be considered to result from an increase in the capacity of and a reduction in resistance to blood flow throughout the entire arterial system from the root of the aorta to the capillaries. There is no evidence to support the postulate that an impairment of skeletal muscle tone or the extravascular mechanism is herein concerned with the fall in blood pressure in these supine animals.

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THE VOLUME FLOW OF RESTING SALIVARY SECRETION

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The term "resting salivary secretion" is used here to mean the salivary secretion which occurs in the absence of any obvious external salivary stimulus. A few investigators (Becks, 1939; White and Bunting, 1936) have previously used this term; and they, and others (Zaus and Fosdick, 1934), have given quantitative data on the volume of this secretion. The data of these authors are either too few or are published in such a form that it is difficult to determine the accuracy with which this measurement or a mean of several such measurements on an individual can be reproduced; in fact, the assumption that the volume of resting salivary flow is a characteristic of an individual could possibly be challenged.

The present data, collected on male students of two classes of sophomore students of dentistry aged 20 to 29 years, are analyzed in relation to this problem in an attempt to contribute to the formulation of a standard technic, and to the establishment of normal limits, for the volume flow of resting salivary secretion.

METHOD. All determinations are carried out in mid-morning or mid-afternoon to avoid as far as possible any effect of meals or hunger on salivary flow. The subjects are requested not to smoke during the ten minutes prior to the determination; they are warned against gum chewing or candy eating previous to the determination. Psychic influences, such as resentment, embarrassment, etc., are avoided. The subject, in a sitting position, bends his head forward to the horizontal and allows the saliva to flow into a 50 cc. beaker placed lightly in contact with the lips, under the opened mouth. Mouth breathing, movement of the tongue, swallowing movements, etc., are avoided. The saliva drains into this beaker for 5 minutes in order to allow time for the establishment of a steady flow, so that the subsequent volume outflow of saliva represents the volume secreted. This beaker is now removed with a sliding motion to take the excess saliva from the lips and a second 50 cc. beaker is quickly substituted. After exactly 5 minutes, the second beaker is removed in the same manner as the first, and the volume is measured in a 10 cc. graduated cylinder after adding 0.1 cc. butyl alcohol to destroy the foam. No correction is made for the volume of alcohol added, since this approximately equals the volume of saliva left in the beaker.

The method used is a rather arbitrary one based to a great extent on a quantity of data obtained with one subject and on the known facts of salivary flow. This general method of measuring total salivary flow has been used by others (Becks, 1939; White and Bunting, 1936). In our use of this method, precautions were taken to avoid stimulation of salivary flow by the passage of air through the mouth by having the collecting vessel (beaker) in close contact with the lips. The time period of salivary collection used results from a compromise

between the obtainment of a volume sufficient for reasonably accurate measurement and the avoidance of restlessness of the subject. The reasons for most of the precautions taken before and during the period of saliva collection are obvious. Some of these precautions are difficult to execute with certainty, since many of these depend upon the co-operation of the subject. This element of uncertainty can be reduced by direct observation of the subject before and during the determination. This was done for two determinations on each of 37 subjects; the values obtained did not differ greatly from those obtained by the subject himself after suitable instruction; however, it is possible that in certain

TABLE 1

Values for the volume of resting salivary flow obtained on 10 subjects (S)

Each figure represents the number of cubic centimeters of saliva collected in a 5 minute period. The values are recorded in the order in which the determinations (D) were made. The mean (M), the standard deviation (S.D.), the standard deviation in per cent (S.D. per cent), and the standard error of the mean (S.E.) are given for each subject.

S / D	W. A.	A. D.	I. G.	M. G.	F. J.	G. L.	G. Q.	R. R.	D. S.	C. W.
1*	1.4	3.0	3.0	1.1	0.3	0.5	0.8	3.6	3.3	1.1
2*	0.4	2.3	4.8	1.0	0.5	0.9	1.0	1.7	2.2	1.6
3	0.6	2.1	2.2	1.3	0.9	0.5	1.5	3.1	3.3	2.4
4	1.3	2.8	1.6	2.5	0.4	0.5	1.2	2.7	2.9	1.7
5	1.5	2.1	1.6	1.2	1.1	0.8	1.0	3.6	2.6	1.3
6	1.0	2.0	2.1	1.9	1.6	0.4	1.2	2.9	2.9	1.7
7	0.7	2.1	2.4	1.6	0.4	1.2	1.3	2.1	2.6	1.8
8	1.2	2.2	3.1	1.5	1.2	0.9	1.0	4.6	2.6	2.0
9	1.8	2.5	1.3	1.6	1.0	0.9	1.2	2.1	2.0	2.4
10	0.7	2.1	2.8	1.7	1.4	1.0	1.2	2.9	1.9	1.8
11	0.7	1.1	2.2	2.1	1.0	1.3	1.0	1.8	2.2	1.6
12	0.9	2.2	3.0	2.1	1.6	0.9	1.5	4.5	1.8	1.1
13	0.9	2.2	2.2			0.5	0.8	2.6	2.7	
14		2.0	1.9			0.5	0.3	2.5		
M	1.01	2.19	2.44	1.63	0.95	0.77	1.07	2.91	2.54	1.71
S.D.	0.41	0.43	0.88	0.45	0.47	0.29	0.30	0.91	0.49	.43
S.D. (%)	40	20	36	28	49	38	28	31	19	25
S.E.	0.11	0.11	0.23	0.13	0.14	0.08	0.08	0.24	0.14	0.12

* These values were taken under the direct supervision of a trained observer.

instances the psychic effect of this direct supervision may have decreased the salivary flow in certain subjects (see table 1). Because of the possibility of the psychic factor influencing the results, most of the data was obtained by the individual subject with a minimum of supervision.

Results. Table 1 gives data obtained on a series of 10 of the subjects for the purpose of estimating the variability of values of resting salivary secretion in each individual subject. This group represents approximately one-quarter of the total number in one sophomore class, and so forms a large sample of this class. This sample was probably not greatly distorted by the method of selec-

tion; for it represents the first 10 students to volunteer as paid subjects. The first three values on these subjects were obtained in the summer and fall and the remainder in the winter of the current school year (1942–1943). The table also gives the mean, the standard deviation, the standard deviation expressed as per cent of the mean, and the standard error of the mean of each series of determinations.

Table 2 shows that the distribution of values around the mean value for each subject of table 1 conforms approximately to the theoretical.

Table 3 gives a limited number of single determinations taken a year apart on a few students. These data indicate that the resting salivary flow does not vary to a much different extent over an appreciable time period than it does over a short period of time (see table 1).

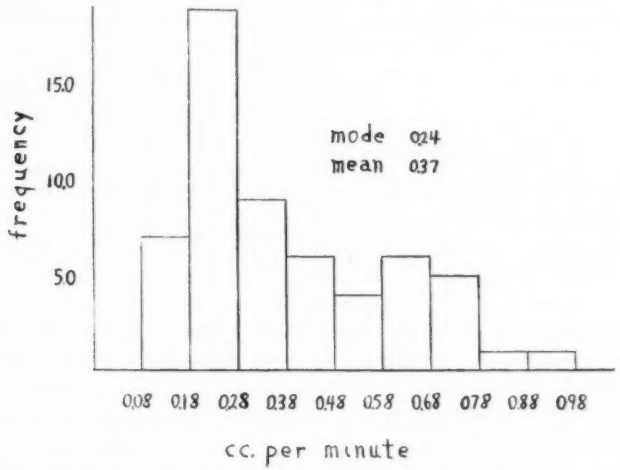

Fig. 1. The distribution of values for the resting flow of salivary secretion obtained on 58 subjects. The values used are the means of two determinations on each subject. The frequency is the number of individuals actually present in each group.

Figure 1 shows the distribution of the means of 2 values of resting salivary secretion of 58 subjects.

Discussion. The variability of values for the volume of resting salivary secretion in the individual subject is quite large (see table 1). From our experience, it is believed that this variability could not be greatly decreased by controlling the experimental conditions more accurately; and, for the present, it is assumed that a rather large variability is inherent in the flow of saliva and that the values obtained represent random samples of the salivary output for the given set of conditions. This assumption is supported by the fact that the standard deviations, when expressed in per cent, are of the same order of magnitude in the 10 subjects studied (see table 1), and the fact that values for each subject are distributed around the mean value in fair agreement with the theoret-

ical distribution (see table 2). Also supporting this is the fact that others (Becks, 1939; Zaus and Fosdick, 1934) have reported considerable fluctuation in the rate of saliva flow in the individual.

Granting this assumption, it is seen that the mean value of salivary flow for a given subject may be determined quite accurately if a sufficient number of determinations are made (see standard errors of the mean values in table 1). Since the mean volume of salivary secretion is sufficiently different amongst the subjects studied, it may be concluded that the mean volume of salivary flow is a characteristic of the individual subject. A few salivary flow values taken

TABLE 2

The distribution of values obtained on each subject (S) about their mean (from table 1)

The interval (I) width is one standard deviation. All values are in per cent. The theoretical distribution is given in the column at the extreme right. The mean of the percentages (M) found in each interval is given in the next to the last column.

I \ S	W. A.	A. D.	I. G.	M. G.	F. J.	G. L.	G. Q.	R. R.	D. S.	C. W.	MEAN	THEO-RET.
+2 to +3	0	0	7	0	0	0	0	0	0	0	1	2
+1 to +2	15	14	0	25	17	14	14	14	15	17	14	14
0 to +1	23	36	29	17	41	43	36	21	46	25	32	34
0 to −1	54	43	57	41	17	36	43	50	15	41	40	34
−1 to −2	8	0	7	17	25	7	0	14	23	17	12	14
−2 to −3	0	7	0	0	0	0	7	0	0	0	1	2

TABLE 3

Values for the volume of resting salivary flow in 5 subjects taken at different times

Each figure represents the number of cubic centimeters of saliva collected in a single 5 minute period.

SUBJECT	FALL 1941	SPRING 1942	SUMMER 1942	FALL 1942
H. H.	1.8	1.8	1.7	1.9
G. L.	0.5	0.9	0.5	0.5
H. R.	2.4	3.2	3.4	2.3
S. S.	1.1	1.5	1.8	1.7
W. W.	3.1		2.9	3.0

over a period of a year on a limited number of subjects give support to this conclusion (table 3).

Some indication of the number of determinations that should be made on a subject in order to know with some accuracy his mean resting salivary flow can be obtained from the data of table 1 by calculating the standard error of the mean from the standard deviation for various numbers of determinations used to obtain the mean by use of the formula S.E. $= \frac{\text{S.D.}}{\sqrt{N}}$. As is well known, the standard error decreases rapidly at first, and then more slowly, as the value of N is increased. Thus it might be expected that if only 4 measurements were taken on each of these 10 individuals, the standard errors would have values of the

magnitude of 10 to 24 per cent of their mean values; while if 9 measurements were made the standard errors would have values of the magnitude of 6 to 17 per cent of their mean values. Conclusions from such a calculation should be applied with some caution however; for it should be realized that the fewer values taken, the greater will be the error due to a single erratic determination. Also greater variability may be found in some subjects than occurs in these individuals who were studied.

The distribution of the means of 2 values for 58 subjects is shown in figure 1. Although the mean of 2 values is certainly an inaccurate measure of the salivary flow of a subject, it is likely that this distribution curve represents in a general way the true distribution of values for these subjects. It is seen that the curve is skewed, the mean and the mode of the distribution curve being respectively 0.37 and 0.24 cc. per minute. The standard deviation was not calculated because of the skewness of the distribution. Resting salivary flow values obtained by the authors mentioned earlier are somewhat higher than the present values. Differences in technic, in experimental conditions, and in type of subject may be responsible for this.

SUMMARY

Using the method described for determining the resting volume of salivary secretion, evidence was obtained that the mean of a sufficient number of determinations represents a value which is reproducible and characteristic of the individual. The distribution of the mean of 2 values of a limited number of subjects is skewed. The modal value is about 0.24 cc. per minute. The lowest mean of two values obtained was 0.08 cc. per minute; and the highest, 0.88 cc. per minute.

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DEVELOPMENT OF HOMEOTHERMY IN SUCKLING RATS

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Perhaps the most useful quantitative index of postnatal *structural* development is increase in size. Among the more interesting indices of *functional* development is increase in what Cannon terms homeostasis (1), the maintenance of a constantly favorable internal environment in the face of external change.

It is generally known that human infants, and young mammals of many other species, do not possess the ability to maintain a constant body temperature; their homeothermic mechanisms are not well developed (2). As Cannon has remarked, homeothermic mechanisms are not required by the developing mammal before birth. At birth, however, survival becomes increasingly contingent upon the exercise of these mechanisms. This report traces the development of homeothermy in the albino rat from birth to weaning at 22 days of age.

METHODS. We investigated the development of ten litters of suckling rats totaling 93 animals. Since intervals of two or more days separated repeated observations on any single individual, so as to avoid the possibility of injury or decreased growth rate due to the experimental treatment, each point in figure 1 represents the average of data from about twenty animals. This procedure may have been the cause of some irregularity in the curve. Records were obtained by placing each subject in a covered container immersed in water at a temperature of 14.7–15.0°C. for fifteen minutes, and then measuring (by means of a thermocouple) the difference between the rectal temperature of the animal and the temperature of the water bath.

RESULTS. Figure 1 shows the development of homeothermy in suckling rats. "Homeothermy" is gauged by the difference between rectal and environmental temperatures, when the environment is cool. Body weights and heat production (oxygen consumption) are also given on the arithlog grid of figure 1 for purposes of general comparison.

At birth the body temperature was only one degree above that of the environment. At the age of one day, the difference between body and environmental temperatures rose to 3°–4°C., and so on, as shown in figure 1. Under the given conditions normal homeothermy for the species was practically fully achieved at the weaning age, 22 days, when the body temperature remained at 37°C. in an environment of 15°C. It is, perhaps, significant that the attainment of full homeothermy comes at an age when the mother is no longer available as a source of warmth.

The central nervous center for the adaptive reaction to cold appears to be

[1] Grateful acknowledgments are made to Professors A. C. Ragsdale (Dairy Husbandry) and Addison Gulick (Biochemistry) for advice and facilities.

located in the hypothalamus (3), and the age curve of homeothermy probably reflects in part the functional development of the heat-regulating center of the hypothalamus. Shivering and other muscle reflexes tending to increase the internal temperature become apparent at age nine to twelve days (4). The development of the endocrines, particularly the adrenals (5) and thyroid (6), may play a rôle in the development of homeothermy. Age changes in homeothermy may be related to age changes in energy metabolism. In this respect it is interesting to note that figure 1 shows similar irregularities in the curves of heat production and homeothermy in the region of nine days. Thus, the age curve

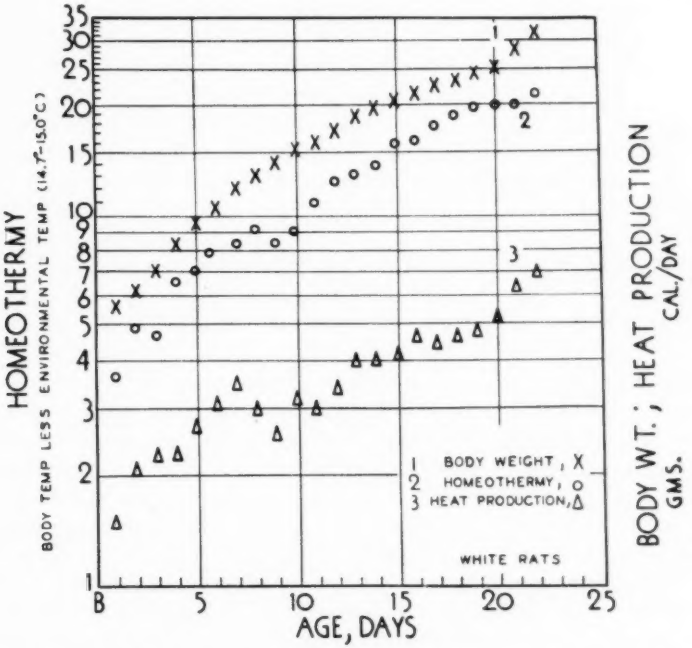

Fig. 1. Age curves of development of homeothermy (curve 2), body weight (curve 1), and heat production (curve 3) in rats.

of homeothermy in figure 1 represents the summation of contributing nervous, vascular, hormonal, and metabolic mechanisms.

SUMMARY

Homeothermy in white rats, as measured by the difference between body and environment temperatures, is practically complete at the weaning age of 22 days. The curves of homeothermy and heat production show similar irregularities in the region of nine days, at which age shivering and other muscular reflexes to cold become apparent.

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THE EFFECT OF MANGANESE AND BORON COMPOUNDS ON THE RAT INCISOR

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The present communication represents a part of a study intended to search for elements which can produce enamel hypoplasia. It was thought that in finding the common qualities of such elements the primary causative factors in the production of enamel hypoplasia could be determined. This study is concerned with the effect of manganese and of boron.

Manganese is present in all living tissues, both plant (1, 2) and animal (3). It is present in the blood in fairly constant concentration (3). Addition of 3.6 per cent manganous chloride ($MnCl_2 \cdot 4H_2O$) to the diet produces moderate to severe rickets (4). At the present time it is the most potent activator of liver phosphatase known (5).

It has been fairly well established that the presence of *boron* is vital for the normal utilization of calcium by plants even when the supply of the latter is adequate (6). Apparently boron is not essential for the normal growth and development of the rat (7).

Since no information is available concerning the effects of manganese and boron on the dental tissues, an investigation into such effects seemed advisable. Special interest was attached to the experiments on the influence of boron following the recent report that a correlation exists between the fluorine and boron contents of natural waters (8).[1]

METHODS AND MATERIAL. The present report is based on studies of the upper incisors of 27 white rats. Thirteen animals were given a single subcutaneous injection of 1 cc. of manganese chloride (C. P.) solution in concentration varying from 1 to 15 per cent (table 1). Eight animals were given a single subcutaneous injection of 1 cc. of a boron containing compound (table 2). Six animals served as controls.

Albino rats used in these experiments were bred Wistar Institute stock and fed dog chow (Purina). Twice a week, lettuce, carrots and dry bread crusts were added to the diet. The animals were kept in a light room—3 to 5 in a cage. The animals were killed by prolonged narcosis with chloroform. Four animals receiving the highest dosage of manganese chloride and one animal injected with a solution saturated with both borax and boric acid died before the end of the experiment (tables 1 and 2).

[1] After the paper had been submitted to the publisher, H. T. Dean, U. S. Public Health Service, kindly called attention to the fact that "apparently there is little consistency in this relationship when viewed over a broad expanse, such as the country as a whole, although in circumscribed geographical-geological areas the relationship may hold."

The specimens were fixed immediately in Zenker-formalin (Maximow) for one day or Bouin's solution for two days. The dissected jaws were decalcified in

TABLE 1

Experimental data and changes of enamel and dentin of 13 rats injected with 1 cc. of manganese chloride solution

NO. AND SEX	AGE AT START	WEIGHT AT START	WEIGHT AT END	CONC. OF $MnCl_2$ SOLUTION	pH	DOSAGE IN MGM. PER KGM.	LENGTH OF EXPER.	ENAMEL HYPOPLASIA	INCREMENTAL LINES	
									Enamel	Dentin
		gm.	*gm.*	*per cent*			*days*			
1 ♂	5 m. 6 d.	235	239	1.0	4.30	42	8	−	−	−
2 ♂	5 m. 20 d.	260	248	2.5	4.20	96	8	−	−	+
3 ♀	2.5 m.	139		2.5	4.20	181	6	+	−	+
4 ♂	5 m. 20 d.	234	216	5.0	4.08	213	8	+	+	+
5 ♀	2.5 m.	136		5.5	3.95	404	6	+	+	+
6 ♂	6 m. 9 d.	237	254	10.0	3.95	417	8	+	±	+
7 ♀	2.5 m.	139		8.0	3.95	576	6	+	+	+
8 ♂	6 m. 9 d.	230	239	15.0	3.76	652	8	−	−	+
9 ♀	2 m.	100		8.0	3.95	808*	6	+	−	+
10 ♀	2 m.	99		8.0	3.95	808	6	+	+	+
11 ♂	2 m.	115		11.0	3.87	956*	1	−	−	−
12 ♀	2 m.	113		11.0	3.87	973*	4	+	−	+
13 ♀	2 m.	106		15.0	3.76	1429*	1	−	−	−

* Indicates that the animals died spontaneously; the others were sacrificed by over anesthesia with chloroform.

TABLE 2

Experimental data and changes in dentin and enamel of 8 rats injected with 1 cc. of boron compounds

NO. AND SEX	AGE AT BEGIN.	WEIGHT		INJECTION OF 1 CC.	pH	BORON PER KILO	DURATION	HYPOPLASIA	INCREMENTAL LINES	
		At begin.	At end						Enamel	Dentin
	months	*grams*	*grams*			*mgm.*	*days*			
14 ♂	1	48	71	5% borax	8.9	39.6	9	−	+	+
15 ♂	1	53	75	1% borax & 1% bor. acid	8.1	40.1	9	−	−	−
16 ♀	1	47	67	5% borax	8.9	40.4	9	−	−	+
17 ♀	1	41	57	5% borax	8.9	46.3	9	−	−	−
18 ♂	1	43	66	1% borax & 1% bor. acid	8.1	49.3	9	+	−	−
19 ♂	1	53	70	5% borax & 4% bor. acid	8.0	168.0	9	−	+	+
20 ♂	1	48	65	5% borax & 4% bor. acid	8.0	185.0	9	−	+	+
21 ♀	1	45		5% borax & 4% bor. acid	8.0	198.0	3*	Not investigated		

* Indicates that the animal died spontaneously; the others were sacrificed by over anesthesia with chloroform.

5 per cent nitric acid, embedded in celloidin and the sections stained with hematoxylin and eosin.

FINDINGS. *A. Injection of manganese chloride.* Manganese chloride solution produced necrosis at the site of the injection in all instances.

Enamel hypoplasia was found in 8 of 9 rats receiving 180 to 975 mgm. $MnCl_2$ per kgm. body weight and investigated 4 to 8 days after injection. In all instances the enamel matrix in the area of the hypoplasia is very thin and wedge-shaped with the base of the wedge toward the incisal end. The hypoplastic area is sharply demarcated against the areas of normal enamel at both apical and incisal ends. The length of the hypoplastic zone varied from 0.3 to 0.6 mm. in the eight animals studied.

The enamel epithelium covering the hypoplastic area shows changes of different degrees, which are apparently not related to the length of the experiment or to the dosage. The enamel epithelium might be formed by small cuboid cells (fig. 1, A), or spindle-shaped cells orientated in different directions (fig. 1, B). In figure 1, C, the incisal covering of the hypoplastic area is formed by cuboid

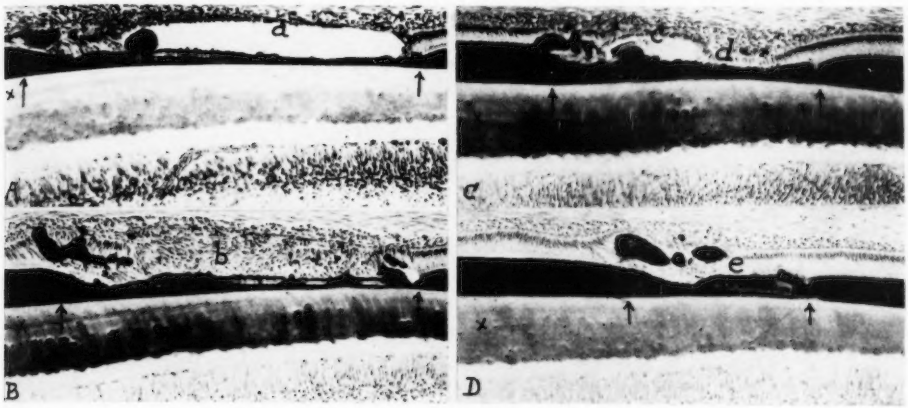

Fig. 1. Single subcutaneous injection of manganese chloride. Variation of changes in the enamel organ covering the enamel hypoplasia (areas between the arrows) and incremental bands in the dentin (*x*) Magnification ×150. The ameloblasts in figure 1A (no. 6) are cuboid (*a*); in figure 1B (no. 10) spindleshaped (*b*); in figure 1C (no. 5) incisally cuboid (*c*) and apically high columnar (*d*); in figure 1D (no. 3) the ameloblasts show minor changes (*e*).

cells, whereas the cells at the apical end are high columnar cells. In figure 1, D, where the hypoplastic area is shorter than in previous cases, most of the ameloblasts covering this area show minor morphological changes.

The photomicrographs demonstrate further differences in regard to the attachment of the enamel epithelium to the hypoplastic area and in regard to the changes of the stratum intermedium and outer enamel epithelium. In figure 1, A and C, the enamel epithelium is detached from the surface of the enamel matrix and the stratum intermedium and outer enamel epithelium can not be distinguished. In the other two cases there is no such detachment and the stratum intermedium and outer enamel epithelium can generally be distinguished (fig. 1, B and D).

In all instances the enamel hypoplasia was covered by an amorphous mass of varying thickness. At the incisal border of the hypoplasia, islands of amorphous

masses are found in the ameloblastic layer. Incremental lines in the enamel matrix were observed incisally to the hypoplasia in some instances (table 1).

Accentuated incremental lines in the dentin (*X* in fig. 1, A, B, C and D), corresponding to the injection were found in all animals receiving 100 mgm. per kgm. or more. They are more distinct in the middle than in the apical and incisal part of the incisor; accentuation was also greater in the labial than in the lingual side.

B. Injection of boron compounds. All animals gained weight. Three animals injected with 5 per cent borax solution showed after 9 days a dry skin ulcer on the site of injection. Possibly this effect is referable to the high pH of this solution (table 2).

Accentuated incremental lines in the enamel have been found in three instances of highest concentration of boron. Only one case (rat 18) of seven investigated showed an enamel hypoplasia.

Four cases of seven showed an accentuated incremental line in the dentin. The line is most distinct at the incisal half of the labial side.

Discussion. All animals injected with manganese chloride in a dosage greater than 180 mgm./kgm. showed enamel hypoplasia. After injection of boron only one animal of seven showed any such lesion and this animal received a comparatively low dosage; from this, one can safely conclude that the hypoplasia in the latter animal was probably unrelated to the injected boron. Enamel hypoplasia is even found, though rarely, in control animals. The observation that boron compounds apparently had no effect on the growth processes of the rat incisor might be significant. The association of boron and fluorine in natural waters (8) suggests that any effects of these waters cannot be attributed to the boron content. In a recent publication (9) it has been shown that boron compounds form a water-soluble complex with riboflavin, and that these preparations are suitable for injection. The findings that are reported herein indicate that the boron content of such preparations would not disturb the development of the teeth.

In most instances the subcutaneous injection of both manganese and boron solutions produces severe lesions at the site of injection leading to an ulcer of the skin. Since enamel hypoplasias were produced in the animals receiving manganese chloride but not in those receiving boron, it may be assumed that the disturbance in enamel formation was due primarily to manganese and cannot be associated with the skin lesion.

The morphologic changes of the ameloblasts in the area of hypoplasia were not constant. The ameloblasts varied from spindle-shaped to cuboid and from cuboid to high columnar. In the case of the single boron animal, the ameloblasts covering the hypoplastic matrix showed no marked morphologic changes. Variations were observed not only in sections from different animals but even in the same specimen. The different morphologic changes of the ameloblasts could not be correlated with the length of the experiments (6 to 9 days) or the dosage (table 1).

In all instances where enamel hypoplasia was found, the matrix underlying

the lesion was cuneiform in shape with the base directed incisally, and sharply bounded against normal enamel. The base of the wedge never attained the full thickness of completed enamel matrix. The hypoplastic area produced by manganese differs markedly from that produced by a single injection of strontium (10). In the strontium experiments, the hypoplasia extends over the entire zone in which the ameloblasts were producing enamel matrix at the time of injection (fig. 2, A). This zone is approximately 2 mm. long. The base of the wedge-shaped hypoplastic area, after injection of strontium, has the thickness of fully developed enamel. When, after the injection, formation of new enamel matrix begins, it is produced only apically to the hypoplastic zone. (*E* in fig. 2A) The strontium hypoplasia, therefore, is sharply bounded only at the apical end. In contrast to these findings, the hypoplasia produced by manganese is much shorter (about 0.5 mm.) and involves only the apical one-fourth of the zone of matrix formation (fig. 2, B). When matrix formation begins after the injection, matrix is not only laid down apically to the hypoplasia

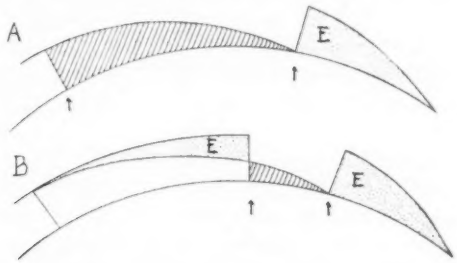

Fig. 2. After single strontium chloride injection (*A*) all ameloblasts forming enamel at the time of injection cease functioning and new enamel (*E*) is formed only apically of the hypoplasia (areas between the arrows). After a single manganese chloride injection (*B*) only the ameloblasts in the apical one-fourth of the formative area are affected. New enamel (*E*) is formed not only apically from the hypoplasia but also incisally.

but also incisally until the matrix has attained a normal thickness, (*E* in fig. 2, B). Therefore, the manganese hypoplasia is not only shorter than hypoplasia produced by strontium but it is demarcated sharply on both its ends by normal enamel. In the case of strontium, all ameloblasts which are actively engaged in the formation of enamal matrix are affected by the drug. On the other hand, in the animals receiving manganese, only a part of such ameloblasts were affected and these in the apical one-fourth of the area where enamel matrix formation was taking place. This varying degree of susceptibility of ameloblasts of different age to various agents may be of significance.

SUMMARY

1. Manganese chloride administered subcutaneously to white rats in dosages greater than 180 mgm./kgm. produces enamel hypoplasia.

2. The lethal dose of manganese chloride for white rats is approximately 800 mgm./kgm.

3. The subcutaneous administration of borax and/or boric acid with a total boron content of less than 200 mgm/kgm. does not cause enamel hypoplasia.

4. The injection of manganese interrupts amelogenesis in the apical one-fourth of the zone of matrix formation. This is in contrast to hypoplasia produced by strontium which extends over the whole formative zone.

5. Various agents may affect ameloblasts in different stages of their life cycle.

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COMPARISON OF SULFOCYANATE WITH RADIOACTIVE CHLORIDE AND SODIUM IN THE MEASUREMENT OF EXTRACELLULAR FLUID[1]

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The apparent volume of distribution of intravenously injected sulfocyanate has been widely used as a measure of the volume of the extracellular fluid (1, 2, 3, 4, 5). The volume of distribution of this substance four hours after injection is fairly reproducible in the individual animal, even though the volume may fluctuate considerably after twenty-four hours (5). The proportion of the body weight corresponding to the water through which sulfocyanate is apparently distributed after four hours varies markedly, however, from animal to animal (1, 3, 4, 5). Often it clearly exceeds the usual extracellular volume of the dog, based on analyses of the water, sodium, and chloride content of whole animals (6). Its identification with the true extracellular fluid volume is, therefore, open to question.

The use of the radioactive isotopes of chloride and sodium, Cl^{38} and Na^{24}, has permitted a new approach to this problem. The chemical behavior of these radioactive isotopes is indistinguishable from that of the naturally occurring isotopes, while the radioactivity of the amounts used for tracer purposes is too small to have a demonstrable biological effect (7). Salts of Cl^{38}, of Na^{24}, and of SCN were therefore injected intravenously into dogs and their respective volumes of distribution followed during the next few hours. Variations in each volume and the relative size of the three volumes with alterations of the true extracellular fluid have been studied in this manner.

PROCEDURE. One adrenalectomized, and two normal lean, female dogs were the principal experimental subjects. Sodium sulfocyanate in 1 per cent solution was injected intravenously from a calibrated burette, followed by a solution containing radioactive Cl^{38} from a calibrated syringe. The latter solution was prepared by dissolving solid LiCl which had just been bombarded with deuterons in the cyclotron. A known amount of a solution of sodium chloride, containing Na^{24}, was injected one to twenty-four hours after the injection of Cl^{38} and SCN, and its distribution similarly determined. At one, two, and four hours after each injection samples of blood were drawn from the jugular vein, while at the same time urine was obtained by catheterization. In the earlier experiments

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it was found that negligible amounts of the substances injected were excreted in the urine during the first two hours after injection. In the later experiments urine was therefore obtained only after four hours. Volumes of distribution of Cl^{38} could only be studied for two hours after injection because of the short half-life of this isotope (37 min.); those of Na^{24} and SCN were studied for four hours.

Volumes of distribution of these substances were determined in the two normal dogs both before and after twelve days of complete deprivation of food and water. In other experiments with these animals, an isotonic solution of glucose was introduced into the peritoneal cavity and permitted to remain there for four hours, at which time an equal amount of fluid was withdrawn. Since this fluid now contained sodium and chloride, the net result of this procedure was the removal of salt without water, and a consequent considerable reduction of extracellular fluid over the next three days. The volumes of distribution of the several test substances were determined before and again three days after this procedure. In the adrenalectomized dog the volumes of distribution of Cl^{38} and of SCN were obtained before and after treatment with desoxycorticosterone acetate and with cortical extract.

METHODS AND CALCULATIONS. Chloride in serum was determined by Hald and Patterson's modification of the Van Slyke method (8), in urine by the method of Volhard and Harvey (9). Sodium in serum was determined by the method of Hald (10), in urine by that of Butler and Tuthill (11). Sulfocyanate in both serum and urine was determined by the method of Crandall and Anderson (1), as modified by Elkinton and Taffel (5).

The concentration of the radioactive isotopes, Cl^{38} and Na^{24}, were determined with a Geiger-Müller counter by a method which has previously been described (12). A sample of the solution injected was diluted until it gave a count in the range of maximum accuracy of the counter; all samples of serum and urine were then diluted until they gave counts in the same general range. Volumes of distribution at any time, $E_{Cl^{38}}$ and $E_{Na^{24}}$, were calculated by the formula:

$$E = \frac{(R_i \times D_i \times V_i) - (R_u \times D_u \times V_u)}{R_s \times D_s \times \text{Donnan factor}} \div W_s$$

where R refers to the count per minute (corrected to zero time for radioactive decay), D to the dilution factor, and V to the volume. The subscript i refers to the solution injected, u to the urine, and s to the serum. W_s is the proportion of water in serum. The Donnan factor used for Cl^{38} was 1.05, that for Na^{24} was 0.95.

The difficulties involved in the calculation of the volume of distribution of sulfocyanate have been discussed in detail elsewhere (5). The theoretical formula for the apparent volume of distribution is:

$$E_{SCN} = \frac{\text{Amount given} - \text{amount excreted}}{\dfrac{\text{Rise in serum concentration}}{\text{Water content of serum}}} \times \text{Donnan factor} \times (1 - \text{fraction bound})$$

The denominator represents the rise in concentration in extracellular water, which in turn is assumed to be identical with the ultrafiltrate of serum. In the sera of the particular normal dogs used in the present study, the concentration in ultrafiltrate was determined directly and was found to be approximately 0.90 × serum concentration (or 0.83 × serum water concentration) (13). Therefore the following simplified formula for the apparent volume of distribution of sulfocyanate, E_{SCN}, was employed in this study:

$$E_{SCN} \frac{\text{Amount given} - \text{amount excreted}}{\text{Rise in serum concentration} \times 0.9}$$

Changes in the volume of the extracellular fluid were also independently calculated from the chemically determined balances of chloride and sodium (14):

$$E_{b_{Cl_2}} = \frac{(E_{Cl_1}38 \times Cl_1) + b_{Cl}}{Cl_2}$$

$$E_{b_{Na_2}} = \frac{(E_{Na_1}24 \times Na_1) + b_{Na}}{Na_2}$$

where:

E_{Cl_1} 38 and E_{Na_1} 24 = distribution volumes of Cl^{38} and Na^{24} respectively before the change,

b_{Cl} and b_{Na} = balances of Cl and Na.

Cl_1 and Cl_2 = initial and final concentration of Cl in extracellular water,

Na_1 and Na_2 = initial and final concentration of Na in extracellular water.

The concentration of Cl and Na in extracellular water, ECW, were calculated from the serum water concentration by the use of a Donnan factor of 0.95:

$$Cl_{ECW} = Cl_s/(W_s \times 0.95),$$
$$Na_{ECW} = (Na_s \times 0.95)/W_s,$$

where W_s = kilograms of water in a liter of serum.

By all these chemical methods, as well as by the volumes of distribution of Cl^{38}, Na^{24}, and SCN, the change in volume of the extracellular fluid was calculated as:

$$\Delta E = E_2 - E_1,$$

where E_2 and E_1 = final and initial volumes respectively.

ΔE as plotted in figure 1 is the average of the differences between the volume of distribution at one and at two hours after injection of the substances.

RESULTS. The essential data are summarized in table 1. These may be analyzed in several ways.

a. *Changes in the volumes of distribution with time.* The single determinations of the volumes of distribution of Na^{24} and SCN after one-half hour were lower than those obtained later, suggesting that equilibrium had not yet been attained.

TABLE 1

Apparent volumes of distribution of Cl^{38}, of Na^{24}, and of SCN; serum concentrations and net balances of Cl and Na

DAY OF EXPT.	CONDITION	HOUR AFTER INJECTION	WEIGHT	E_{Cl}^{38}	E_{Na}^{24}	E_{SCN}	$E_{b_{Cl}}$	$E_{b_{Na}}$	WEIGHT	E_{Cl}^{38}	E_{Na}^{24}	E_{SCN}	$E_{b_{Cl}}$	$E_{b_{Na}}$
			Dog 1. Normal						Dog 2. Normal					
			kgm.	*liters*	*liters*	*liters*	*liters*	*liters*	*kgm.*	*liters*	*liters*	*liters*	*liters*	*liters*
1	Hydrated	½	11.21			4.38			11.43			3.69		
		4	10.98			4.65			11.28			4.13		
5		½	10.84	2.76					11.40	2.58				
6		½	10.88		2.20				11.52		2.78			
		2	10.62		3.09				11.48		3.30			
		4	10.61		2.95				11.40		3.00			
19	Hydrated	1	11.04	2.86		4.18			11.38	2.70		4.12		
		2	11.01			4.19			11.34			3.97		
		4	10.94			4.22			11.28			3.99		
20		1	11.20		3.16				11.42		2.76			
		2	11.14		3.19				11.40		3.23			
		4	11.12		3.18				11.54		3.13			
27	Hydrated	1	11.50	2.94		3.89			11.52	2.86		3.75		
		2	11.70	3.27		4.29			11.42	2.83		3.91		
		4	11.50			4.25			11.44			3.83		
28		1	11.36		3.52				11.44		2.97			
		2	11.36		3.35				11.44		2.80			
40	Deprived of water and food 12 days	1	8.90	2.17		3.31			9.14	2.09		2.99		
		2, 1	8.88		2.75	3.60	2.32	2.81	9.06		2.60	3.08	2.32	2.26
		2	8.86		2.60		2.63	2.65	9.04		2.52		2.30	2.10
54	Rehydrated and fed	1	10.20	2.68		3.93	2.79		10.50	2.36		3.54	2.91	
		2	10.14	2.63		3.99	3.14		10.42	2.36		3.53	2.88	
		1	10.14		3.60				10.36		3.44			
		4, 2	10.10		3.60	3.96			10.36		3.28	3.63		
		4	10.00		3.50				10.32		3.35			
75	Hydrated	1	10.34	2.62		3.67			11.06	2.33		3.56		
		2	10.26			3.78			11.00			3.59		
		1	10.22		2.89				10.82		2.71			
		2	10.20		3.04				10.76		2.83			
78	Salt withdrawal 3 days	1	9.34	1.64		3.01	2.11	2.18	9.80	1.68		2.80	1.70	1.80
		2, 1	9.30		2.40	3.44		2.34	9.74		2.03	2.77		1.93
		2	9.32		2.55				9.68		2.16			

DAY OF EXPT.	CONDITION	HOUR AFTER INJECTION	E_{Cl}^{38}	E_{SCN}	DAY OF EXPT.	NET BALANCE		SERUM CONCENTRATION			NET BALANCE		SERUM CONCENTRATION		
						Cl	Na	H_2O	Cl	Na	Cl	Na	H_2O	Cl	Na
	Dog 3. Adrenalectomized					Dog 1. Normal					Dog 2. Normal				
			liters	*liters*		*m. Eq.*	*m. Eq.*	*gms. per liter*	*m. Eq. per liter*	*m. Eq. per liter*	*m. Eq.*	*m. Eq.*	*gms. per liter*	*m. Eq. per liter*	*m. Eq. per liter*
1	No treatment	1	2.04*	2.84*	28			938	108.1	142.7			944	113.3	144.9
11	DOCA + cortin	1	2.62†	3.29†	40	−62	−80	932	112.8	149.6	−55	−74	934	117.0	156.2
24	DOCA + cortin	1	2.65†	3.47†	54	+33		938	104.5	142.2	+39		944	106.4	137.5
33	DOCA	2½	2.76†	3.64†	75			939	108.9	140.0			942	111.2	137.5
					78	−86	−103	929	98.5	137.3	−124	−133	920	85.4	120.5

* Serum water content assumed to be 910 grams per liter.

† Serum water content assumed to be 930 grams per liter.

The volumes of distribution of these two substances sometimes changed between the first and second and again between the second and the fourth hours after injection. These changes were never large, however, and were not consistent in direction. It therefore seems more reasonable to ascribe such variations to technical difficulties or to true changes in the volumes through which the substances were distributed, than to lack of equilibrium between plasma and interstitial fluid after one hour. Of the four determinations of the volume of distribution of Cl^{38} at one and again at two hours after injection, three were identical and one was slightly greater after two hours, suggesting that for this substance, too, equilibrium is usually complete after one hour.

b. *Relative magnitudes of the volumes of distribution of Cl^{38}, Na^{24} and SCN.* Under all conditions of hydration the volume of distribution of Cl^{38} was uni-

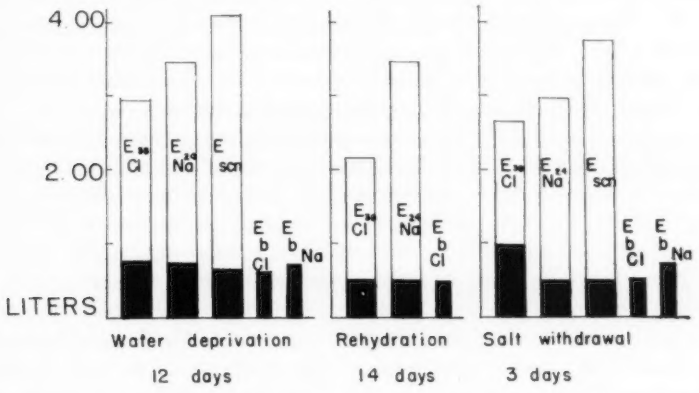

Fig. 1. Comparison of measurements of changes in extracellular fluid volume in dog 1. Blank columns represent the initial absolute magnitude of the volumes of distribution of Cl^{38}, Na^{24}, and SCN, before change has occurred. Black columns represent the changes, $\Delta E_{Cl^{38}}$, $\Delta E_{Na^{24}}$, ΔE_{SCN}, ΔE_{bCl} and ΔE_{bNa}. In the first and third experiments the changes were negative, in the second, positive. The results with dog 2 are similar.

formly the smallest, and that of SCN the largest of the three substances. In terms of per cent of body weight, the average volumes of distribution of the three substances in dogs 1 and 2 when normally hydrated were: 24.7, 27.6, and 35.6 for Cl^{38}, Na^{24}, and SCN respectively.

A statistical analysis of the difference between the volumes of distribution of the three substances was carried out. $E_{Cl^{38}}$ was taken as a standard for reference. In the three dogs, hydrated and dehydrated, the mean of 34 individual ratios of $E_{Na^{24}}$ to $E_{Cl^{38}}$ was 1.25, with a standard deviation of $\pm$ 0.16. Similarly, of 38 individual ratios of E_{SCN} to $E_{Cl^{38}}$ the mean ratio was 1.52 with a standard deviation of $\pm$ 0.16. The difference between E_{SCN} and $E_{Cl^{38}}$ is therefore somewhat more significant statistically than that between $E_{Na^{24}}$ and $E_{Cl^{38}}$.

c. *Effect of changes in hydration on the volumes of distribution of Cl^{38}, Na^{24}*

and SCN. Changes in the distribution volumes of Cl^{38}, of Na^{24} and of SCN were compared under conditions known to produce changes in the volume of extracellular fluid. Changes were induced in each of the two normal animals, once by water deprivation, and once by salt withdrawal by intraperitoneal glucose solution. The changes were also measured by chloride and sodium balances over the three periods. Despite large differences in the initial distribution volumes of the three substances, the changes as measured in two to five ways were always in the same direction and were of approximately the same magnitude (fig. 1). Similarly the changes in the volumes of distribution of Cl^{38} and SCN, produced by treatment in the adrenalectomized dog, dog 3, over 33 days were + 0.72 liter for $\Delta E_{Cl^{38}}$ and + 0.80 liter for ΔE_{SCN}.

Although the changes as measured by the different methods agree for the most part, there are some discrepancies which appear to bear no relation to the initial absolute magnitude of the distribution volumes or to any one of the substances used. That is, there are occasional single differences from the average value of as much as −48 and +74 per cent. The data on changes are too few to be submitted to a satisfactory statistical analysis.

Discussion. The results of these experiments are perhaps best understood if it is recalled that the extracellular fluid volume is the summation for the whole organism of a large number of particular fluid phases in many different kinds of tissue. The distributions of chloride, sodium, and sulfocyanate undoubtedly vary from one tissue to another. Sulfocyanate is definitely known to enter the cells of certain tissues, such as erythrocytes and gastric mucosal cells (1, 2), and indirect evidence has been presented that it probably enters the cellular phase of other tissues as well (5). Sodium is present in the dog erythrocyte (15) and in the cells of striated muscle (6). Cohn and Cohn (16) have shown that Na^{24} rapidly penetrates dog erythrocytes. Manery and Bale (17) in an extensive study of the penetration of Na^{24} into the different phases of many types of tissues conclude that although its entrance into some sodium-containing cells is slow, in others, especially secretory cells, the entrance is rapid. Greater cellular penetration in certain tissues must explain the larger volumes of distribution of sodium and of sulfocyanate.

Other things being equal, in experiments of this type the substance giving the smallest volume of distribution is logically the best measure of the extracellular fluid. Chloride, on this basis, should be the substance of choice. But chloride itself is known to enter erythrocytes, and hence must give a measurement which is somewhat too large. Chloride is also known to be present in the cells of connective tissues, pyloric mucosa, and testes (18). However, as Manery and Haege (19) have shown that in the case of rat testes and rabbit pyloric mucosa, Cl^{38} in the first few hours penetrates only the sodium or extracellular phase, intracellular distribution of chloride in these tissues probably does not occur over the two hour interval of these experiments. At present we can only state that any substance, such as sulfocyanate, whose volume of distribution exceeds that of radioactive chloride, is unsatisfactory in the absolute measurement of extracellular fluid. This does not preclude its use as a relative measure of

changes in the extracellular fluid volume, and our evidence on the whole supports the validity of the use of sulfocyanate for this purpose.

However, these experiments indicate that serial determinations of the volume of distribution of some single test substance may give misleading values for changes in the extracellular fluid volume. If such changes are to be studied, it is desirable to check changes in the distribution volume of sulfocyanate or of any other test substances by chloride and sodium balances.

The conclusion should not be drawn that the ratios of the absolute magnitudes of the volumes of distribution of Na^{24} and SCN to Cl^{38} are 1.25 and 1.52 respectively in all or any dogs. These data were obtained from only three dogs. A review of all the determinations of the distribution of sulfocyanate in normal hydrated dogs in this laboratory since the present SCN method has been in use shows that, in terms of body weight, the average value for E_{SCN} was 34.6 per cent with a maximum of 42.3 per cent and a minimum of 26.7 per cent. These figures are from 29 determinations, made one to four hours after injection in 12 dogs, and include those reported in this paper and in a previous paper (5). This wide range of values agrees well with those found by other workers; values of 27 to 36 per cent, 23.0 to 42.5 per cent, and 21.2 to 37.0 per cent of the body weight having been reported by Crandall and Anderson (1), Gregersen and Stewart (3), and Mellors et al. (4), respectively. In view of this wide variation the volume of distribution of SCN and its relation to that of Cl must vary considerably in different dogs.

Although fairly constant volumes of distribution of SCN have been obtained at one to four hours after repeated injections over the course of a few weeks, there is some evidence that over a longer period of time the cellular phases into which SCN penetrates rapidly during the first four hours may become relatively saturated after repeated injections. Thus in table 1 E_{SCN} fell gradually over an eleven week period from 43.3 per cent of the body weight to 35.5 per cent in dog 1 and from 36.6 per cent to 32.2 per cent in dog 2. Six months after the first of these determinations E_{SCN} was 30.4 per cent and 26.7 per cent of the body weight in these two dogs respectively.

Kaltreider, Meneely, Allen and Bale (20) have studied the relation of the volumes of distribution of radioactive sodium and of sulfocyanate in man. These workers found that both volumes, after a sharp rise during the first three hours, reach a fairly constant level during the next nine hours. The magnitudes of the two volumes were very similar and approximated 25 per cent of the body weight. The difference between their results in man and our results in the dog may be due to the difference in species.

SUMMARY AND CONCLUSIONS

In three dogs under varying conditions of hydration the absolute magnitude of the apparent volume of distribution of sulfocyanate always exceeded that of radioactive sodium, which in turn exceeded that of radioactive chloride.

In the hydrated normal dogs Cl^{38}, Na^{24}, and SCN were distributed through

approximately 25 per cent, 28 per cent and 36 per cent of the body weight respectively.

In spite of these differences in the absolute magnitude, changes in the volume of the extracellular fluid as measured by each of the three substances and by chloride and sodium balances were usually of about the same magnitude; occasionally unexplained discrepancies appeared.

The volume of distribution of sulfocyanate is therefore not a satisfactory absolute measure of the volume of the extracellular fluid. Under most conditions, however, it may be a useful relative measure, i.e., changes in its volume of distribution may reflect changes in extracellular fluid.

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THE CENTRIFUGAL COURSE OF WALLERIAN DEGENERATION

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In a previous report (Rosenblueth and Dempsey, 1939) Parker's (1933) conclusion was confirmed that Wallerian degeneration is faster at more central than at more peripheral regions of a severed axon. The question arises whether the faster rate of degeneration near the section of the nerve is determined by the section itself, or whether it depends on a gradient intrinsic to the axons. This study attempts to answer that question.

METHOD. In cats under ether or nembutal anesthesia the peroneal nerves were cut at one or more of the following regions: the hip, the middle of the thigh, and the knee, before the nerve begins to distribute its branches. These sections were made with aseptic precautions. Care was taken not to cut the arteries which accompany the nerves, in order to preserve the blood supply.

Two to four days later, the cats were anesthetized with dial (Ciba, 0.7 cc. per kgm., intraperitoneally). The degenerating peroneals were dissected from hip to knee. Depending on whether or not the section at the middle of the thigh had been made, one (10 to 12 cm.) or two (5 to 6 cm.) stretches were thus available for study from each side of the animal. After crushing the ends of each stretch they were placed on electrodes in a moist chamber.

The nerves were stimulated maximally by brief condenser discharges with a frequency of 10 to 30 per sec. This frequency was regulated by a thyratron which tripped the stimuli and also the sweep of the cathode-ray oscillograph.

The electric responses were led monophasically or diphasically to the amplifier. Capacity-coupled amplification was employed, with a time constant of 0.2 sec. The responses were photographed from the standing waves in the face of the oscillograph. Each picture corresponded to from 3 to 6 superimposed responses. The description of the position of the stimulating and recording electrodes is given with the corresponding experimental results.

The areas underlying the monophasic responses were measured as follows. The records were projected through a photographic enlarger and were drawn on light cardboard (filing cards). They were then cut out and were weighed to $\pm$ 5 mgm. The average weight was about 250 mgm. Since the sweep was not strictly linear (see figs. 1*f* and 3 D) a correction of these weights was made by dividing their value by the speed of the sweep at the region where the peak of the spike was located. The latter figure was closely proportional to the area of the responses.

The observations were made mainly on the A fibers of the nerves and only incidentally on B axons.

[1] Fellow of the J. S. Guggenheim Memorial Foundation.

RESULTS. A. *The degeneration gradient in nerves with a single cut at the hip.* In the study of Rosenblueth and Dempsey (1939) the existence of this gradient was concluded from a comparison of the amplitude of the spike potentials of the degenerating trunks with those of normal controls when the nerves were stimulated at different regions from center to periphery. In the present observations more accurate methods were used, as follows.

If in normal nerves the recording electrodes are kept in a fixed position on intact and crushed regions, respectively, the amplitude of the spikes decreases gradually as the stimulating cathode is moved away from the recording region (fig. 1A). As is well known, this decline of amplitude is due to increasing

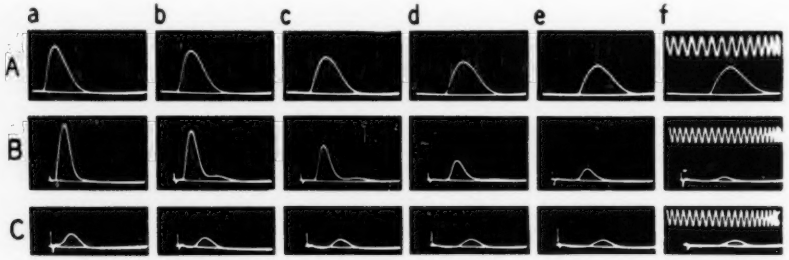

Fig. 1. Changes of amplitude of maximal monophasic spike potentials with variable conduction distance. The time of stimulation is indicated by the stimulus artifacts in B and C. In A this artifact was too small and does not appear. Its position was fixed and occurred shortly after the beginning of the sweeps. The speed of the sweeps is calibrated for A, B and C, respectively by the sinusoidal waves (5,000 cycles) at the top of *f*. In these and other records the horizontal line corresponds to the level of the oscillograph for the resting nerve.

A. Normal peroneal. Record from the peripheral end (interelectrodal distance 1.5 cm.). Distances between the stimulating cathode and the proximal recording electrode: *a* to *f*, from 2 to 7 cm. by 1 cm. steps.

B. Degenerating peroneal cut both centrally (hip) and peripherally (knee) 4 days previously. Records as in A.

C. The same degenerating nerve as in B, but records from the central end. Amplification increased. Conduction distances from 2.5 to 7.5 cm.

temporal dispersion. The areas of the recorded response, however, decrease only slightly with increasing conduction distance (fig. 2A).

Series of observations were made with variable stimulated points and with fixed recording electrodes at both the central and the peripheral ends of the degenerating nerves. Typical results were similar to those illustrated in figures 1 and 2. When the record was peripheral both the amplitude and the area of the spike potentials declined markedly with increasing conduction distance—i.e., with stimuli applied progressively closer to the central end. When the record was central the amplitude decreased only slightly and the areas remained practically constant as the conduction distance was increased.

The gradient of the areas with peripheral recording was steeper the more advanced the degeneration. Thus, with stimuli moved toward the central end, only a slight decrease of area was seen in nerves cut 2 days previously, while

the decrease was very pronounced in nerves studied 4 days after section. The sigmoid shape of the middle curve in figure 2A is typical. The position of the steeply ascending portion varied with the degree of degeneration. Thus, in 3-day nerves that portion was close to the central regions while in 4-day nerves it was located in more peripheral regions.

The gradient of responses supports the inference of a gradient of degeneration. With a peripheral record the responses decrease when the stimuli are moved away from the record because less and less fibers are stimulated—i.e., because

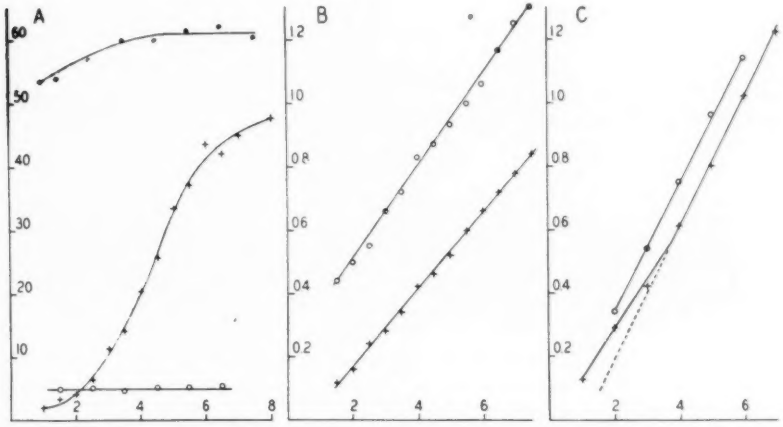

Fig. 2. A. Areas of maximal monophasic spike potentials with variable stimulated region. Ordinates: areas in arbitrary units. Abscissae: distance (in centimeters) between the stimulating cathode and the central cut of the nerves.

Upper curve (dots). Normal peroneal. Record from peripheral end.

Middle curve (crosses). Peroneal, 4 days after 2 cuts had been made, at the hip and knee, respectively. Records from the peripheral end.

Lower curve (circles). Same nerve as in B. Records from the central end.

B. Uniformity of the conduction velocity along a normal peroneal. Ordinates: latencies in milliseconds. Abscissae: conduction distances in centimeters. Lower curve: beginning of responses. Upper curve: peak of the spike potential of the A fibers.

C. Conduction velocity of fibers along a degenerating peroneal (4 days). Ordinates and abscissae as in B. Lower curve: beginning of responses with peripheral recording. Upper curve: same, but central recording.

more and more fibers have degenerated to a stage where they do not conduct. With a central record the responses do not vary much because many of the few recording fibers functional in that region will be activated throughout the nerve. Impulses set up in the functional peripheral part of other fibers will not reach the central region. The S shape of the middle curve in figure 2A indicates that there is a relatively short stretch of nerve separating the highly degenerated, nonfunctional central region, from the slightly degenerated, functional peripheral part.

An alternative method of testing the nerves was to move from center to

periphery, or vice versa, all 4 electrodes, stimulating and recording, keeping the respective distance constant—e.g., 1 cm. interelectrodal recording and stimulating distance, and 1.5 cm. conduction distance. Except for the positions in which one of the recording electrodes was at a crushed end, the records were diphasic.

When this procedure was used in normal nerves the responses were about 30 per cent smaller from more peripheral than from more central recording regions. This difference suggests that the amplitude of the recorded spike potentials is smaller in the distal than in the central portions of the nerves. Standard monophasic records supported that inference. The source of this asymmetry will be discussed later.

Similar tests made on degenerating nerves led to opposite results—i.e., the responses were larger at the more peripheral than at the more central regions. These results again support the inference that at a given time after central section, more fibers are degenerated towards the centers than towards the periphery.

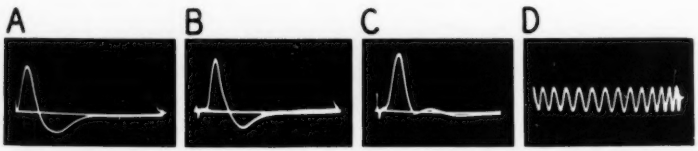

Fig. 3. Changes of diphasic responses in degenerating peroneals. The recording electrodes were 1 cm. apart, and placed at least 2 cm. away from the cut ends. The conduction distance was 2 cm.

A. Normal nerve. The record begins at the end of the stimulus artifact.

B. Degenerating nerve (4 days). Record from a relatively peripheral part. Stimulus applied centrally. The stimulus artifact precedes the response.

C. Degenerating nerve (4 days). Record from a relatively central part. Stimulus applied peripherally.

D. Five thousand cycles.

A further difference between normal and degenerating nerves was the following. As seen in figure 3A the 2 phases of the diphasic normal electrogram are prominent. Indeed, the areas of these 2 phases are approximately equal. This equality obtains whether the stimuli are applied at a central or at a peripheral region, with respect to the recording electrodes. In degenerating nerves, on the other hand, while the diphasic electrograms obtained from central stimulation resembled the normal responses (fig. 3B) those recorded with peripheral stimulation were asymmetric. The 1st phase was greater than the 2nd one. The records tended to become monophasic (fig. 3C).

A final difference between normal and degenerating nerves appeared when conduction-velocity measurements were carried out. In normal nerves the conduction velocity of the fastest fibers was uniform throughout the trunks tested (lower line in fig. 2B). In addition the latency of the peaks of the spike potentials varied uniformly with increasing conduction distance (upper line, fig. 2B), indicating that the rate of conduction for the average A fibers was uniform. The

velocity of propagation was the same in measurements made with peripheral and with central recording, respectively.

While the graphs correlating latency of response with conduction distance were straight lines when the record was from the central end of the degenerating nerves, they could deviate significantly from the straight line when the record was from the peripheral end. This was true not only of the latency of the beginning of the responses (fig. 3C), but also of that of the peak of the A spike potential. The deviation was more striking when the degeneration was more advanced—i.e., it was seldom found before 3 days, but was the rule after 4 days of degeneration.

The conduction velocities measured in degenerating nerves were sometimes faster than normal when degeneration was not very advanced (up to 105 m/sec. in some of the 3 day nerves; cf. table 1). This confirms similar observations of Rosenblueth and Dempsey (1939). With more advanced degeneration the

TABLE 1

Conduction velocities (m/sec.)

The rows indicate the propagation velocity of the beginning of the responses (*fastest* fibers) and of the *peak* of the A spike potential (average A fibers). In the columns the symbols mean as follows: 3 and 4 days, time of degeneration; C and P, central or peripheral recording; *c* and *p*, stimulation of a relatively central or peripheral region. Each figure is the average of from 5 to 11 measurements in different nerves. There was little scatter except for the columns corresponding to 3 days of degeneration.

	NORMAL NERVES	3 DAYS		4 DAYS		
	C and P	C	P	C	P*c*	P*p*
Fastest	82	75	83	55	49	73
Peak	62	63	65	45	44	72

rate of propagation was slower than normal throughout the nerve, but it was slower in the central than in the peripheral regions (table 1).

The irregular lower line of figure 2C is readily explained by the interpretation that the larger fibers, which conduct more rapidly, are totally degenerated in the central portions, while some of them are still functional in the peripheral regions. The two slopes in the line thus probably denote two sets of fibers, not a change of velocity in the same elements.

B. *The gradient in nerves with central and peripheral cuts, at hip and knee.* As illustrated in figures 1, 2 and 3, all the tests described in the previous section yielded results in these nerves similar to those obtained with only one cut at the hip. In some animals the double section was made on one side while only a central cut was made on the other. A comparison of the two nerves showed only minor quantitative differences. It may be inferred, therefore, that the peripheral cut at the knee does not modify the centrifugal course of Wallerian degeneration.

C. *The nerves with a central and a middle cut, at hip and middle thigh.* The purpose of these observations was to see whether a single gradient would obtain

for the nerve as a whole, from hip to knee, or whether each cut would set-up two similar gradients, one for the upper and the other for the lower segment of the nerve. In other words, the question was whether or not the degree of degeneration would be the same at the central end of the two segments divided by the middle cut—i.e., immediately below the cut at the hip and below that in the middle.

The results indicated clearly that a single gradient was present for the nerve as a whole. Thus, after 2 or 3 days of degeneration the upper segment usually showed a marked centrifugal gradient while the lower one did not show it—i.e., the lower segment was practically undegenerated throughout its extent, while the upper one showed extensive degeneration toward the central end.

After more prolonged degeneration (3 to 4 days), the A fibers of the upper segment as a rule were almost totally degenerated while a sizable fraction of them were functional in the lower portion.

The amplitude of the monophasic responses recorded with a standard procedure was invariably greater at the lower central region (below the middle cut) than at the upper one (below the cut at the hip). The responses at about 1 cm. below the middle cut were only slightly smaller than those at about 1 cm. above this section.

It may be inferred, therefore, that an additional middle cut does not modify the course of degeneration of the nerve as a whole consequent to a higher section at the hip.

Discussion. Some considerations of the structure and properties of normal peroneal nerves, in the regions studied, are necessary for the evaluation of the results on the degenerating trunks. Specifically, the question arises whether this part of the peroneal may be considered a homogeneous, uniform trunk, or whether there is a systematic anatomical change in the A axons from center to periphery. This question is suggested by the observation that the amplitude of responses is smaller at more peripheral than at more central parts of normal nerves (p. 250). A change in the average diameter of the axons along the nerve would result in a change of the amplitude of the spike potential (see Gasser and Grundfest, 1939).

The observations of Eccles and Sherrington (1930) oppose the view that there is a significant change of the average diameter size of the axons in the regions of the nerves tested. They found only a slight degree of branching, and hence a slight decrease of diameter, of motor axons measured between 9 and 62 mm. away from the gastrocnemius muscle. This branching was more marked in the regions close to the muscle than in more central parts of the nerve. The changes of diameter of the axons in the nerves studied here would therefore be practically negligible.

In support of the inference that the trunks studied are uniform are the measurements of conduction velocity illustrated in figure 2B. The latencies of the peaks of the spike potentials may be taken as an accurate basis for measurements of average conduction velocity. If the diameter of the axons decreased at the periphery their conduction velocity would become slower, as is well known. The

plot of the latencies against the conduction distance would then yield a graph which would deviate from a straight line. The straight lines in figure 2B indicate, therefore, that the conduction velocities of both the fastest and the average A fibers in the nerves remained uniform throughout the trunk. They indicate, therefore, that there is no significant progressive decrease of diameter of axons from center to periphery in this part of the nerves.

It may be concluded from this argument that the smaller amplitude of responses recorded peripherally, as compared to those recorded centrally, is not due to a change in diameter of the fibers. An alternative, more likely, explanation is that as the nerve begins to organize into its several peripheral branches there is an increase in the relative amount of fascia surrounding the axons. This fascia acts as a shunt which decreases the recorded potentials. A further corollary of the foregoing discussion is that the gradient of degeneration revealed by the data is not a consequence of a non-uniform structure of the axons.

Since the blood supply of the nerves was carefully preserved at the time of the sections, it may be concluded that the centrifugal gradient of degeneration is not determined by changes in the irrigation of the tissue.

That the gradient is not determined by the section of the nerves is indicated by the fact that it was not modified when several cuts were made, instead of only a central section (figs. 1 and 2, p. 251). The lack of influence of additional sections excludes the diffusion at the cuts of some substance into or out of the axons as a possible factor for the asymmetry in the progress of degeneration. The results lead to the inference that the centrifugal course of degeneration is due to a polarization or gradient which is intrinsic to the axons. The nature of this gradient is obscure at present. An obvious working hypothesis is that it is metabolic, so that more central regions of axons burn up more rapidly than more peripheral parts. Other hypotheses are possible, however, and only future study will clear the problem.

It may be pointed out that the demonstration of an asymmetry in axons, and even the knowledge of the source of this asymmetry, does not and will not clarify the reason for Wallerian degeneration. The factor or factors responsible for the degeneration of an axon separated from its cell-body and nucleus may be entirely independent of the conditions which determine the time course of that degeneration.

The relative monophasicity of responses recorded from two uncrushed points in the degenerating nerves when the stimuli are applied peripherally (fig. 3C) is attributed to the presence of axons which are functional at the region in contact with the recording electrode proximal to the stimulated part, but which are not functional at the region of the distal recording lead, because of the more advanced central degeneration. As a corollary of this interpretation it may be inferred that non-conducting parts of a degenerating axon behave like injured parts of normal axons with respect to the recording of the action potential from other normal or less degenerated regions.

The present observations confirm previous reports that larger fibers degenerate more rapidly than smaller axons (see for references Spielmeyer, 1929). Thus,

the abnormal prominence of the B spike potentials in nerves with an advanced degeneration, as compared with normal trunks (cf. fig. 1 A and B) indicates more degeneration of A than of B axons. Similarly, as already mentioned (p. 249), the broken lower line of figure 2C is best explained by the assumption that some of the large, fast fibers are entirely degenerated in the central region of the nerves at a time when smaller, slower axons are still functional.

SUMMARY

The peroneal nerve of the cat is shown to have A axons of uniform diameter between the hip and the knee (p. 253). Wallerian degeneration of that nerve follows a centrifugal course—i.e., central portions degenerate more rapidly than peripheral regions (pp. 249, 250). This gradient of degeneration is uninfluenced by cuts additional to the central section at the hip (figs. 1 to 3; p. 252). The gradient, therefore, is not determined by the cuts, thus excluding the possibility of diffusion of some substance out of or into the axons as a factor. The gradient does not depend on the blood supply to the nerve (p. 253). It reveals a longitudinal asymmetry intrinsic to otherwise uniform axons.

It is confirmed that larger fibers degenerate more rapidly than smaller axons (p. 254).

Monophasic action potentials may be recorded from a functional to a degenerated region of an axon (p. 253, fig. 3).

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AN ANALYSIS OF THE THIOCYANATE METHOD FOR DETERMINING THE DISTRIBUTION OF THE BODY FLUIDS

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Since the introduction of thiocyanate by Crandall and Anderson (4) as a substance which is distributed mainly in the extracellular fluids, the method has been used on several occasions for measuring changes in extracellular fluids (11, 12, 14, 15). Attempts at standardization of the method have been carried out by a number of investigators (8, 9, 13). As a result of these investigations the thiocyanate method is appreciated as a valuable adjunct in studying the body fluids under experimental conditions, and the method is generally accepted as valid as far as relative changes are concerned. In some recent studies (1, 2, 10) we have attempted to use the method and have ecountered some difficulties in repeated determinations of available fluid (for solution of thiocyanate). The present study therefore attempts to evaluate the method for approximation of extracellular fluid volume changes and to standardize the method for repeated determinations.

METHODS. Dogs were used as the experimental animal. Anesthesia was usually not employed, but in a few instances pentobarbital sodium intravenously was used. Thiocyanate was administered as 2.5 or 5 per cent solution of the potassium salt. The solution was measured into a syringe by means of a volumetric pipette attached to the syringe tip by a short segment of small-bore rubber tubing. The solution was given by way of the jugular vein and the syringe was rinsed twice with blood to insure complete emptying. Blood samples were taken from the jugular vein at 10 to 15 minute intervals during the first hour, at 20 to 30 minute intervals in the second hour and at variously longer intervals thereafter. Thiocyanate was determined according to the method of Lavietes, Bourdillon and Klinghoffer (9), except that adoption of the method to the Lumetron photo-electric colorimeter was made. We have found the color produced by ferric nitrate reagent to fade within a short period of time, and for this reason the color is produced in each sample separately and read in a uniform time, that is, within 30 seconds after the addition of ferric nitrate. The small amount of thiocyanate present in normal control plasma makes it necessary to make a correction, especially when reinjection of thiocyanate for repeated determination is carried out. To avoid the error, we carry out the procedure on control plasma and use this for the transmission standard instead of distilled water. Plasma thiocyanate determinations were carried out in duplicate. These usually checked within 0.1 mgm. per 100 cc. Urine thiocyanate determinations were carried out by the same procedure as for plasma with the exception that the transmission standard was urine-trichloracetic acid filtrate diluted with an equal volume of distilled water. Sodium determinations were made in duplicate by the method of Butler and Tuthill (3).

Diffusion time. Crandall and Anderson found the diffusion time to be around one hour in dogs. Stewart and Rourke (13) found the plasma level to stabilize in about 30 minutes in man. In 22 dogs in the present study the diffusion time was found to average between 47 to 67 minutes (the average times of the next to last, and last determinations, after which subsequent readings were stable). The maximum time in the series was 67 to 89 minutes. No correlation between diffusion time and size of dog or thiocyanate level was apparent.

Disappearance of thiocyanate. Crandall and Anderson report an average rate of disappearance of 0.08 mgm. per 100 cc. per hour. In 23 dogs used in this study the average rate of disappearance was found to be 0.15 mgm. per 100 cc. per hour as determined over a period of 24 hours. The difference between this figure and that obtained by Crandall and Anderson, if significant at all, may be attributed to different average plasma thiocyanate levels in the two studies. This rate of disappearance was found to vary considerably in different animals, the range being 0.04 to 0.41 mgm. per 100 cc. per hour. To some extent this variation depended upon the concentration of thiocyanate in the extracellular

TABLE 1

Comparison of total disappearance of thiocyanate from extracellular fluid with total urine excretion

EXPERIMENT	TIME	BLOOD LEVEL	DISAPPEARANCE RATE	TOTAL SCN DISAPPEARANCE IN EACH EXPT.	TOTAL AMOUNT SCN EXCRETED IN URINE
	hours	*mgm./100cc.*	*mgm./100 cc./hr.*	*mgm.*	*mgm.*
1	24	12.4 -8.8	0.17	144	21
2	6	7.25-5.7	0.29	36	7.3
3	25	11.3 -7.6	0.16	153	18.5
4	24	8.15-6.95	0.07	38	29
5	17½	10.6 -7.15	0.20	160	16

fluid so that an average per cent disappearance is somewhat more accurate in estimating the rate of disappearance. This percentage factor, in this series of 23 dogs, was found to be 2.3 per cent per hour.

Disposition of thiocyanate. No attempt was made in this study to establish the exact location of the thiocyanate ion as it disappeared from the extracellular fluid. However, some suggestion as to the disposition of the ion may be derived from observations on the following experiments. In 5 dogs quantitative determination of urine thiocyanate over a period of about 24 hours was compared with the amount which disappeared from the original available fluid, which latter was assumed to remain constant in volume. As shown in table 1, the results of this study indicate that the amount disappearing from this original available fluid far exceeds that quantity which is eliminated in the urine during the same period. According to these findings, the repeated determination of available fluid by subtracting that thiocyanate excreted in the urine from the total amount originally injected as was done by Elkington and Taffel (6) would give erroneous results. This may account for the unreliability of the thiocyanate method for

measuring a given portion of the body fluid over an extended period, as found by these authors, since false high values would be obtained by this method of correction. These data would seem to indicate, in accord with the findings of Elkington and Taffel, that thiocyanate rapidly diffuses into one portion of the body fluids and slowly enters another phase of the body fluids, probably the cells.

Relation of urine excretion of thiocyanate to plasma level. A progressive increase in thiocyanate concentration in the plasma was produced in 4 dogs by continuous intravenous administration of thiocyanate. Urine collections were taken at intervals and blood samples were simultaneously obtained. A distinct correlation of urine and plasma thiocyanate concentration was detected by this means although the urine level was always definitely lower than the level in the plasma. Thus, with increased plasma levels, one may expect a more rapid urine excretion. This, at least, partly explains the higher rates of disappearance found in the animals with higher plasma levels in the first part of this study.

Optimum thiocyanate dosage. The toxic and lethal levels of thiocyanate in the plasma vary with different animals. Rapid injection of doses of 40 to 75 mgm. per kilo may cause death. On the other hand levels of 30 mgm. per 100 cc. have frequently been attained without obvious untoward symptoms. In order to avoid toxic or lethal effects the dosage should not exceed 30 mgm. per kilo. of body weight. The optimum value in the plasma from the technical standpoint is around 8 mgm. per 100 cc. which is approximately attained by the administration of 25 mgm. per kilo of body weight in the dog.

The use of the thiocyanate test in detecting changes in the extracellular fluid. In order to test the accuracy of reinjection of thiocyanate, nine normal dogs were used. The original available fluid was determined by a single injection. In this determination precaution was taken to insure complete diffusion and correction was made for disappearance either by actually determining the disappearance rate in individual dogs or by using the factor 2.3 per cent disappearance per hour as discussed above. After completion of the first determination a second determination of available fluid was made by reinjection of thiocyanate. By taking the difference between the levels attained after reinjection and that existing before reinjection, a second calculation of available fluid was made. The results of single injection and reinjection in normal dogs in which no change in extracellular water was produced are compared in table 2. It is noticeable in the results that there is definite variation, which we consider to represent the culminative vicissitudes of the test. The errors are on both the positive and negative side, that is, neither method is consistently higher or lower. The greatest per cent of error from the first determination is 5.8 per cent and average error is plus or minus 3.6 per cent. In table 3 are presented the data on the use of the thiocyanate method in detecting changes in extracellular fluid volume by causing an increase of extracellular volume in two dogs and a decrease in three dogs. The increase in extracellular fluid volume was brought about by ligation of both ureters and injecting 1000 cc. of normal saline intravenously. The extracellular fluid was caused to decrease by the method used by Gilman (7) and Darrow and Yannet (5) which consists of the intraperitoneal injection of 5 per

cent glucose. In these two groups of experiments available fluid was calculated *1*, from a single injection of thiocyanate, determining the rate of disappearance from the percentage factor 2.3 per cent per hour, or, in some dogs, the disappearance rate was previously determined; *2*, by reinjection of thiocyanate; and

TABLE 2

Comparison of available fluid determination from original injection with available fluid as determined by reinjection of thiocyanate

EXPERIMENT	1ST EXCELL. DETN.	REINJECTION	ERROR	PER CENT ERROR
	cc.	*cc.*	*cc.*	
1	3780	3580	−200	−5.3
2	2490	2470	−20	−0.8
3	3880	3960	+80	+2.3
4	3760	3980	+220	+5.8
5	4520	4390	−130	−2.9
6	3080	3040	−40	−1.3
7	2620	2760	+140	+5.3
8	3980	4120	+140	+3.5
9	4300	4650	+250	+5.8
Average				3.6

TABLE 3

Table illustrating changes in extracellular water in superhydration and extracellular dehydration

EXPERIMENT	PROCEDURE	ORIGINAL AVAILABLE FLUID	BY SCN DISAPPEARANCE	PER CENT CHANGE	BY SCN REINJECTION	PER CENT CHANGE	BY SODIUM METHOD	PER CENT CHANGE
1	Ureters ligated; 1000 cc. 0.9 per cent NaCl intravenously	2520	3310	+31.6	3650	+45.2	3460	+37.6
2	Ureters ligated; 1000 cc. 0.9 per cent NaCl	2870	3700	+28.6	4030	+40	4076	+42
3	400 cc. 5 per cent glucose intraperitoneally. Removal of 400 cc. fluid with 28 mgm. SCN and 1.01 grams Na	2270	1845	−18.4	1960	−13.0	2067	−9.0
4	750 cc. 5 per cent glucose IP. Removal of 840 cc. fluid with 29 mgm. SCN and 2.13 grams Na	3317	3040	−8.4	3067	−7.5	3083	−7.1
5	1000 cc. 5 per cent glucose IP. Removal of 1060 cc. fluid with 34 mgm. SCN and 2.69 grams Na	4595	4000	−13.0	3927	−14.5	4250	−5.1

3, extracellular fluid volume changes were calculated from changes in plasma sodium concentration. In the dogs receiving 5 per cent glucose intraperitoneally correction was made for the thiocyanate and sodium which diffused slowly into the peritoneal fluid. In the table it is shown that results by the three methods

are fairly concordant and are in accord with the expected type of fluid redistribution brought about by the methods used.

Discussion. According to results obtained in the present study confirmation is given to the use of the thiocyanate method in determining a partition of the body fluids as available fluid for solution of thiocyanate. Certain precautions are necessary for proper utilization and evaluation of the test. In order that the optimum blood level shall be produced, approximately 25 mgm. per kgm. of body weight should be administered. In each case the diffusion time should be carefully determined by taking samples of blood, 30 minutes, one hour, one and a half hours, and two hours after the first injection.[1] Diffusion is usually complete about one hour after the injection, but a single sample one hour after the injection is not safe because in a few dogs diffusion is not complete then. It is preferable that the disappearance rate be determined in each case by taking a sample 3 to 4 hours after the injection (before this time the decrease of thiocyanate will be too small to measure accurately) and correction for disappearance should be made in calculating the available fluid. The use of a disappearance factor, in this study found to average 2.3 per cent per hour, yields less accurate results. This disappearance factor is usable however, in view of the roughly quantitative nature of the test.

Determination of available fluid subsequent to the original determination may be made by one of two methods: *1*, by determining the rate of disappearance during the control period and calculating the available fluid from the thiocyanate level resulting from the original injection after changes in extracellular fluid have occurred, and *2*, by reinjection of thiocyanate.

The relatively high thiocyanate levels resulting after a second or third injection, and the possibly more rapid disappearance of thiocyanate when the plasma level is elevated cause us to favor the first method, that is, the disappearance method. In studying changes in extracellular water it would seem preferable to check the thiocyanate method, preferably a single injection with disappearance and reinjection procedure combined, with the method of determining extracellular fluid changes by variations in sodium concentration.

The exact fate of thiocyanate was not ascertained in the present study. The demonstrated fact that a considerably greater quantity of thiocyanate disappears from the available fluid than is excreted in the urine during a given time would strongly suggest that thiocyanate continues to enter the cells even after diffusion has been completed.

SUMMARY

1. Optimum dosage of thiocyanate to determine available fluid volume is 25 mgm. per kilogram of body weight, yielding a thiocyanate concentration of around 8 mgm. per 100 cc. of plasma.

2. The diffusion time varies but ranges around one hour. The time should be

[1] In recent experiments we have been giving the thiocyanate to the animal on the day preceding the experiment, taking samples at intervals on the day of injection, and the next morning taking a sample in order to calculate the rate of thiocyanate disappearance.

ascertained in each determination by serial sampling. The disappearance rate averages 2.3 per cent per hour but should also be determined in each instance and correction made accordingly.

3. Reinjection determinations of available fluid check within 6 per cent of the original determination. The disappearance method with a single injection is advocated as the preferable method for determining changes in extracellular water volume subsequent to the original determination. This method should be checked by reinjection of thiocyanate and sodium determinations.

4. In determining experimental extracellular water changes, reasonably concordant results are obtained with the thiocyanate method and the sodium method. The thiocyanate method is considered to be a satisfactory method for roughly measuring changes in extracellular water. Changes in the extracellular water volume in excess of 6 per cent probably have a definite significance by the method.

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HEART RATE IN TAMED AND UNTAMED RATS

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In the course of a previous experiment (5) it was found necessary to establish the normal heart rate of the rat. This rate, it was found, depended on whether the animals were tamed or untamed. This paper presents an analysis of the differences found between the tamed and untamed rats of both sexes.

The heart rate was recorded by a Davis electroencephalograph; clip electrodes were attached ventrally and dorsally through the axis of the heart.

A miniature replica of the conventional Pavlov frame was constructed for recording the heart rate of the adult rats. The animal was kept in the frame by means of straps just behind the forelegs and in front of the hind legs. The floor of the frame is movable and serves a double purpose—it allows for adjustment to the size of the animal, and it is of aid in the training procedure. In the first few days of training, if the animal struggles too much, it displaces the floor, and consequently is left suspended by the two straps—a situation which the rat soon avoids by ceasing to struggle.

In the course of the investigations reported here, the 130 animals used were classified as follows:

1. Tamed (gentled). (a) Thirty days old. Seventeen animals in this group were handled every day from birth. They were held in the experimenter's hand while heart records were taken.

(b) One hundred-twenty days old. Twenty-seven animals in this group were handled from birth and were fastened in the Pavlov frame daily for two weeks before heart recording was begun on the 120th day.

2. Untamed (non-gentled). (a) Thirty days old. Sixty-nine animals, unhandled except for marking, comprised this group. On the 30th day heart records were taken while the animal was held in the experimenter's hands.

(b) One hundred-twenty days old. Seventeen animals, unhandled except for marking, made up this group. On the 120th day the animals were placed in the Pavlov frame and heart records taken.

Water and excess food were always available to both groups.

The two groups were thus equated with regard to the procedure employed on the 30th and 120th days, except that one group was tamed and the other untamed. In addition to the determination of heart rate, the weight of the rats was recorded. Tables 1, 2 and 3 present the results of the data analyzed on these days.

In 3 of the 4 groups the heart rate of the untamed animals was significantly higher than that of the tamed animals. That a true base line of heart rate had been reached for the tamed 120-day-old animals is indicated by the fact that the heart rate average for these 27 animals on the 120th day did not differ signif-

TABLE 1

Comparison of heart rates in tamed and untamed rats at 30 and 120 days of age

SEX	30-DAY			120-DAY		
	Tamed	Untamed	Critical ratio*	Tamed	Untamed	Critical ratio
Male	355 ± 35.6 (N:10)	496.5 ± 57 (N:32)	9.6	422 ± 30.4 (N:16)	506.7 ± 35 (N:10)	7.1
Female	413 ± 45.5 (N:7)	491.7 ± 43 (N:37)	4.2	484 ± 18 (N:11)	483 ± 44.2 (N:7)	.20

* This is an index of the reliability of a difference between 2 measures. A critical ratio of 3 or more indicates that the obtained difference is statistically significant, *i.e.*, the chances are greater than 999 in 1000 that a true difference exists.

TABLE 2

Sex differences in heart rate of tamed and untamed 30-day-old rats and of tamed and untamed 120-day-old rats

AGE	HEART RATE					
	Tamed group			Untamed group		
	Male	Female	Critical ratio	Male	Female	Critical ratio
30 days	355 ± 35.6 (N:10)	413.2 ± 45.5 (N:7)	2.8	496.5 ± 47.1 (N:32)	491.7 ± 43 (N:37)	.4
120 days	422 ± 30.4 (N:16)	484.2 ± 18 (N:11)	6.7	506.7 ± 34.8 (N:10)	483 ± 44.2 (N:7)	1.2

TABLE 3

(*a*) *Differences in weight of tamed and untamed 30-day-old animals and tamed and untamed 120-day-old animals (grams)*

SEX	30-DAY			120-DAY		
	Tamed (1)	Untamed (2)	Critical ratio	Tamed (3)	Untamed (4)	Critical ratio
Male	41.3 ± 8.5 (N:10)	51.1 ± 10.5 (N:32)	2.9	265.7 ± 23.2 (N:16)	303.5 ± 23.1 (N:10)	4.2
Female	43.2 ± 6.6 (N:7)	49.8 ± 11.2 (N:37)	2.0	202.5 ± 13.4 (N:11)	229.4 ± 12.2 (N:7)	4.3

(b) Critical ratio sex differences in wt. col. 1 = 0.5 (not significant).
Critical ratio sex differences in wt. col. 2 = 0.5 (not significant).
Critical ratio sex differences in wt. col. 3 = 9.0 (significant).
Critical ratio sex differences in wt. col. 4 = 8.6 (significant).

icantly from the average heart rate of these same animals based on recordings taken from the 120th to the 134th day inclusive. These rates were 447 for the

1 day period and 451 for the total 15 day period. The procedure of taming outlined above then allows for a reliable 1 day sampling.

The untamed females were less affected by the recording situation than were the untamed males.

Sex differences are present in the tamed animals but not in the untamed animals. Sex differences, however, disappear in the tamed group when recordings are taken under conditions of stress. The latter point was illustrated when the tamed animals of 120 days of age which showed a sex difference with a critical ratio of 6.7 were reintroduced to the Pavlovian frame after a 50-day absence. The situation now, judging by struggling behavior and defecation, was a stressful one and analysis of the data of recordings taken for a 5-day period failed to reveal any sex difference. The rates of both sexes were approximately 475. This disappearance of sex differences may be said to be due mainly to the different action of the male and female heart under stress, the former reaching a higher rate while the females apparently were unaffected. If male and female heart rate gained proportionately, sex differences would still be observable. The fact that they do not results in the males reaching a level approximately equal to that of the females with a consequent disappearance of previously noted sex differences. It was also noted (table 1) that while both untamed male age groups showed a significant difference from the corresponding tamed animals only one of the two female groups showed that difference. These two facts then might suggest a sex difference in regard to sensitivity of heart rate.

Fluctuation in heart rate. This was calculated by analyzing the 30-second period into five 6-second periods and considering the maximum change between any two periods as the fluctuation. No significant differences were found in any of the cross comparisons.

In three of the four comparisons the untamed rats were significantly heavier than tamed rats of the same age and sex. Sex differences in weight were proportional in the tamed and untamed animals for the two age groups.

DISCUSSION. Hammett (2) in 1921 reported, in this Journal, on the mortality rate of two groups of rats in the 48 hours following thyroparathyroidectomy. For the "standard rats" the mortality was 79 per cent of a group of 90; for the "experimental rats," 13 per cent of a group of 96. The "standard rats" referred to the stock albinos, animals rarely handled up till the time of operation. The "experimental rats" were described as animals which had had constant handling and petting. Hammett's "standard rats" then correspond to the untamed rats of the present study, and his "experimental rats," to the tamed rats. Hammett suggests the following hypothesis to account for the difference in mortality rate. The excitement of the standard rats on being handled led to a high muscular tone with a resulting high metabolic rate and more rapid formation of toxic compounds. The experimental rats, on the other hand, maintained a low muscular tone and lower metabolic rate and a less rapid rate of toxin formation. As a result the experimental (tamed) animals survived in larger numbers. The findings of the present study showing differences in heart rate between tamed (experimental) and untamed (standard) animals supplement and support

Hammett's hypothesis, the higher heart rate of the untamed animal implying a higher metabolic rate. This differentiation in heart rate between tamed and untamed rats may also have some bearing on the findings of Humphrey and Marcuse (3) that the untamed rats are more susceptible to convulsive seizures due to auditory stimulation.

Sex differences in metabolic rate, weight of hypophysis, blood coagulation time, etc., have been found in the rat (1). Thus it is not surprising that sex differences in heart rate should exist. The lack of recognition of this difference in the available literature on the subject may be a result of the masking effect on this difference caused by the differential reaction of male and female heart to conditions of stress implied in the recording situation. Schafer (6) states that while the pulse frequently is greater in women than in men, this difference almost disappears if men and women of equal stature are compared. In the present investigation sex differences in weight were present in both the tamed and untamed 120-day-old group. The differences were proportional for the two groups with the males being the heavier. Sex differences in heart rate, however, were found only in the tamed group. In the 30-day group sex differences in weight were not present for either the tamed or untamed groups, but again sex differences in heart rate were found for the tamed group only (tables 2 and 3b). Weight then does not appear to be significantly related to heart rate. This finding, together with the unusual sequence of development of heart rate found in the rat, *low* at birth with subsequent increase, (4), may suggest an atypical heart action system.

SUMMARY

1. Heart rates of untamed 30- and 120-day-old rats were significantly higher than heart rates of tamed rats of the same sex and age in three of four comparisons. This higher rate was more marked in the males.

2. Sex differences noted in the tamed group were not present in the untamed group or in the tamed group when under stress.

3. Untamed animals were significantly heavier than tamed animals of the same sex and age in three of four comparisons.

4. No significant differences in fluctuation of heart rate were found in any cross comparison of groups.

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DEMONSTRATION OF ANTITHROMBOPLASTIC ACTIVITY IN NORMAL AND HEMOPHILIC PLASMAS

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The study of blood clotting has concerned itself to a large extent with the transformation of prothrombin into thrombin and of fibrinogen into fibrin. This emphasis, on what must constitute, under ordinary conditions, the later phases of the changes in the blood after it leaves the vessels has been encouraged by the view that normal "cell free" plasma contains all factors needed for coagulation (1). Though this may be true, the point is often overlooked that the *time* at which coagulation begins and the *rate* of its development are directly influenced by the degree and extent of the changes in shed blood *preceding* the inception of clotting.

It is generally agreed that a clot accelerating substance, thromboplastin, released from tissue cells or the formed elements of shed blood is responsible, in a large measure, for the rapid activation of prothrombin. The mechanism whereby prothrombin is activated remains obscure; the rate and extent of activation is somehow related to the amount of thromboplastin available (2, 3, 4). The following experiments may help to throw additional light on this mechanism. The evidence to be presented appears to indicate that normal human "cell free" plasma *collected with especial precautions*, has the property of reducing the clot accelerating action of aqueous extracts of brain tissue, when plasma and extract are incubated before recalcification. This natural clot decelerating quality of fresh human plasma shall be designated "antithromboplastin activity," or merely "antithromboplastin." Hemophilic plasma possesses an antithromboplastin activity several times greater than normal. This excess is probably the *primary* cause of the pronounced delay in the inception of coagulation of hemophilic blood.

METHODS AND REAGENTS. Unless meticulous care is taken in the collection of blood and separation of plasma little or no activity may be demonstrated. For this reason the various technical steps will be described in detail. Venous blood is collected, preferably from a fasting subject, swiftly (average rate of collection no less than 1 cc. each 2 sec.) through sharp, wetted 18 gauge needles, into syringes loaded with the required amount of anticoagulant. All bubbling should be avoided, and the blood should be gently mixed with the anticoagulant within 30 sec. after it began to be drawn into the syringe. The blood is centrifuged immediately at 3,000 RPM for 45 minutes, the upper $\frac{2}{3}$ of the plasma carefully removed *at once* with paraffin coated droppers, placed in similarly coated tubes, and tested within one hour after its separation. Occasionally, in blood with a high per cent cell volume, centrifugation for 45 min. at 3,000 RPM

does not suffice to rid the plasma of platelets. As additional precaution, a platelet count (1–20 dilution) on the plasma should be done, to make certain that it does not contain more than 4,000 true platelets per mm^3. A 0.129 M sodium citrate solution (3.8 grams of $Na_3C_6H_5O_7 2H_2O$ in 100 cc. distilled H_2O) is used as anticoagulant in the proportion of 1 part of the solution to 9 parts of blood, when the per cent cell volume is 40 ± 5. This will yield a plasma with a citrate concentration between 0.0187 and 0.0214 M (0.55 and 0.63 gram per cent). Recalcification of 0.3 cc. of such plasma (containing 1.6 to 1.9 mgm. of citrate) with 0.1 cc. of a solution containing between 0.7 and 1.0 mgm. of $CaCl_2$ will yield, fairly constantly, optimal (minimal) coagulation times. Care must be taken, however, that the proportions of citrate to calcium are not greatly upset by wide variations in the per cent plasma volume in a given specimen. In order to maintain uniform citrate-calcium ratios when standard calcium solutions are used for recalcification, one must *add* to the blood 0.16 cc. of the citrate solution for every 10 units (or fraction) *below* 35, of the red cell hematocrit; a similar amount should be subtracted from 1 cc. of the citrate solution to be added to the blood, for each 10 units (or fraction) of the red cell hematocrit *above* 45. If the per cent cell volume has not been determined in advance, the amount of $CaCl_2$ required to give a minimal coagulation time for a given mixture of plasma and diluted thromboplastin must be established by trial. As will be shown later, 0.1 cc. of a $CaCl_2$ solution between 0.06 and 0.09 M will nearly always produce minimal clotting times when added to 0.3 cc. of plasma of 0.017–0.022 M citrate concentration (0.5 to 0.65 gram per cent).

The following standard thromboplastin solutions are used: 1. *Concentrated:* 0.85 per cent NaCl extract of acetone dried adult human brain prepared by the Quick method (5). Each 0.1 cc. of this solution should clot 0.1 cc. of normal human recalcified citrated plasma in 11–12 sec.; this time should not be perceptibly altered by incubation of solution with plasma, in these proportions, before recalcification. 2. *Dilute* thromboplastin solutions. *Solution A:* prepared by diluting 0.025 cc. of the concentrated saline brain extract, measured in a serological pipette, with 10 cc. of 0.85 per cent NaCl. Each 0.1 cc. of this solution contains 0.25 $mm.^3$ of the concentrated thromboplastin and should clot 0.3 cc. of optimally recalcified normal citrated human plasma in 50 ± 10 sec. *Solution B:* prepared by diluting 0.033 cc. of a 1–10 dilution of the concentrated saline brain extract with 10 cc. of 0.85 per cent NaCl. Each 0.1 cc. of this solution contains 0.033 $mm.^3$ of the concentrated thromboplastin and should clot 0.3 cc. of optimally recalcified normal, citrated, human plasma in 100 ± 10 sec. The dilute solutions should be fresh; they begin to lose potency after about 2 hours' standing at room temperature. Recalcification is carried out with 0.1 cc. of a $CaCl_2$ solution of varying molar concentration, according to the respective *total* amount of citrated plasma used. If a minimal clotting time on 0.3 cc. of plasma is obtained with 0.1 cc. of a $CaCl_2$ solution other than 0.074 M, the concentration of the $CaCl_2$ for other quantities of plasma must be correspondingly adjusted.

The amounts and order of addition of the reagents are as follows: *Tube 1*

(without incubation): 0.1 cc. diluted thromboplastin, 0.1 cc. $CaCl_2$, 0.3 cc. plasma. *Tube 2* (with incubation): 0.1 cc. diluted thromboplastin, 0.3 cc. plasma, mix, incubate, then add 0.1 cc. $CaCl_2$.

When the effect of a certain agent (heat, dilution, etc.) is being studied, the distribution of amounts and order of addition are as follows: *Tube 1* (without incubation): 0.2 cc. *treated* plasma, 0.1 cc. *fresh* plasma, then rapidly 0.1 cc. $CaCl_2$ (proper M concentration) and 0.1 cc. diluted thromboplastin. *Tube 2* (with incubation): 0.2 cc. *treated* plasma, 0.1 cc. diluted thromboplastin, mix, incubate, then *rapidly* 0.1 cc. $CaCl_2$ (proper molar concentration), and 0.1 cc. *fresh* plasma.

In control tubes the treated plasma is replaced by an equal volume of 0.85 per cent NaCl, the order of additions and rest of procedure remaining the same. In this manner the clotting system $CaCl_2$-fresh plasma is used as an "indicator" of the extent to which incubation with the *treated* plasma (or salt solution) has affected the clot accelerating power of the thromboplastin. The order of additions is so arranged as to allow no opportunity for contact of the thromboplastin with the plasma before recalcification, except when so designed.

Prothrombin is measured by Quick's method (5) using the acetone dried human brain extract. Unless otherwise stated the prothrombin content of all plasmas is 100 per cent of normal. The rate of clotting of plain recalcified citrated plasma (designated throughout these experiments as the "Plasma Clotting Time") is estimated by adding 0.1 cc.of $CaCl_2$ (proper M concentration) to 0.3 cc. of plasma. All incubations and clotting time determinations unless otherwise stated are carried out at 37.5 $\pm$ 0.5°C. in glass tubes 7 mm. internal diameter and 3 cm. in length. The evidence in support of the conclusions in this paper was selected from a group of 351 experiments on blood from dogs, rabbits and men (normal and hemophilic).

1. *Incubation of plasma with thromboplastin solutions.* A diminution in the clot accelerating action of certain thromboplastin solutions may be observed as early as after one minute of incubation with normal plasma (fig. 1). A clear antithromboplastin effect is observed only with solutions of a given clot accelerating power (those containing 1.0 $mm.^3$, 0.25 $mm.^3$ or 0.033 $mm.^3$ of concentrated thromboplastin per 0.1 cc.). A maximum effect seems to be reached after about 5 min.; between that time and 10 min. no further important changes seem to take place. Hemophilic plasma differs from normal in that: 1, it exerts a clot decelerating effect even on potent thromboplastin solutions (10 $mm.^3/cc.$); 2, its effect on the same solution is more pronounced, and 3, within certain limits, grows more intense as the solution becomes weaker.

Since most of the inactivation of thromboplastin by plasma apparently takes place in the first 3 min. of incubation it would be natural to expect that, when the thromboplastin is so diluted as to produce a clotting time approximating that of recalcified plasma without thromboplastin, little or no difference would be shown between the incubated and unincubated specimens. In such instances, the coagulation of the recalcified unincubated plasma itself proceeds at a rate sufficiently slow, to allow most or all of the inactivation to take place during

that period; preliminary incubation with the thromboplastin for 5 min. will, therefore, result in little addition to the amount of thromboplastin inactivated. A similar effect is observed when coagulation of normal plasma is made more prolonged by increasing the *volume* of the plasma while holding the volumes of thromboplastin and $CaCl_2$ fixed. When the clotting times goes beyond 120 sec. the antithromboplastin effect gradually diminishes until a point is reached when the incubated specimens clot faster than the unincubated. In order to

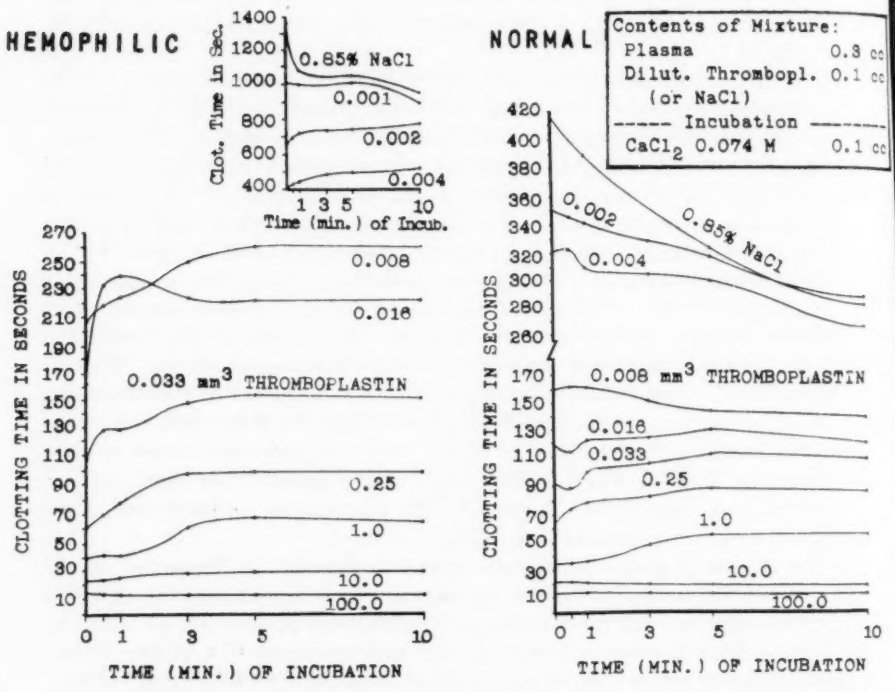

Fig. 1. Effect on the clotting time of hemophilic and normal plasma of incubation for various periods with solutions of thromboplastin of decreasing potency, before recalcification. The numbers adjacent to each curve designate the potency of the thromboplastin solution used, expressed in terms of the actual amount in cubic millimeters of concentrated saline brain extract in each 0.1 cc. of the solution.

detect, by the incubation method, maximum decelerating effects in normal plasma, a thromboplastin solution that produces a clotting time of about 50 sec. seems best. For comparing the activity of normal plasma with that of hemophilics a clotting time of about 100 sec. is desirable. This presupposes equal prothrombin concentrations in the 2 plasmas. "Cell free" oxalated plasmas, separated from blood collected as specified, behave in this respect substantially like citrated plasmas.

In table 1 are analyzed statistically the extent of the clot decelerating action

of the "cell free" plasmas of 51 normal and 6 hemophilic men on the dilute thromboplastin solutions A and B. When using the stronger thromboplastin (sol. A), one may expect in 68.2 per cent of determinations on normal subjects, prolongations of the clotting time of the incubated plasma above that of unincubated, of the order of 12.5 to 43.1 sec.; an equivalent percentage of determinations on hemophilic plasma should yield prolongations of 32 to 55.6 sec. When using the weaker thromboplastin (sol. B) a prolongation of the order of 8 to 29.2 sec. only may be expected in normal plasma, while hemophilic plasmas may yield prolongations of 49.6 to 71.7 sec. As indicated by the values of p, hemophilic plasma exerts an effect significantly greater than that exerted by normal plasma against the weaker thromboplastin solution; against the stronger solution, no statistically significant difference exists between the 2 plasmas.

The longer coagulation time of the incubated plasma-thromboplastin mixture is not due to a diminution in prothrombin, since no difference may be observed

TABLE 1

Significance of differences in antithromboplastin activity between normal and hemophilic plasmas, using 2 types of thromboplastin

TYPE OF THROMBOPLASTIN	NORMAL INCREASE IN CLOT. TIME OF INCUB. OVER UNINCUB.			HEMOPHILIC INCREASE IN CLOT. TIME OF INCUB. OVER UNINCUB.			DIFFER. $\pm \sigma$ DIFF.	$\frac{\bar{x}}{\sigma DIFF.}$	p^*
	Mean ($\pm\sigma$)	Max. Min.	No. of observ.	Mean ($\pm\sigma$)	Max. Min.	No. of observ.			
	sec.			*sec.*			*sec.*		*per cent*
Sol. A	27.8 $\pm$15.3	44 13	59	43.8 $\pm$11.8	92 23	29	16 $\pm$19.3	.82	41
Sol. B	18.6 $\pm$10.6	45 1	38	60.7 $\pm$11.09	114 40	35	42.1 $\pm$15.29	2.75	0.6

* Probability of the difference between the 2 plasmas being due to sampling.

between the prothrombin content of unincubated plasmas and those incubated with dilute thromboplastin solutions for the periods of time stated above. Likewise, incubation of the plasma and thromboplastin solution separately, or of the plasma with 0.85 per cent NaCl before recalcification, either does not alter the clotting time significantly or shortens it, when compared with unincubated controls. Moreover, if the thromboplastin solution is pipetted down carefully on the surface of the plasma and the two are allowed to incubate, without mixing, much less prolongation of the clotting time is observed than when the thromboplastin and plasma are mixed before incubation (table 2). Restricting the site of the reaction between the two liquids to their interface, accounts perhaps for the diminished effect.

2. *Calcium and antithromboplastin.* The importance of maintaining uniform citrate/calcium ratios in these studies may be appreciated when the effect of various amounts of sodium citrate and $CaCl_2$ on antithromboplastin activity is studied.

Normal or hemophilic blood (9 cc.), collected as specified, was drawn into 2 syringes loaded with 1 cc. of a solution of either 0.064 M (1.9 per cent) sodium citrate in 0.4 per cent NaCl or 0.129 M (3.8 per cent) sodium citrate. The bloods were then centrifuged at 3,000 RPM for 45 min. and the respective amount of citrate in each plasma calculated from the percent cell volume. One sample of blood was allowed to stand at 5°C. for 24 hrs. before the plasma was separated by centrifugation. The activity of the normal plasmas was tested on thromboplastin solution A, that of hemophilic plasmas on sol. B, using $CaCl_2$ solutions of varying molar concentration.

With 0.02 M citrate plasmas, minimal clotting times and an antithromboplastin activity of uniform degree are obtained on recalcification with 0.06–0.074 M $CaCl_2$ solutions; under-citrated, 0.01 M plasmas may not manifest a comparable degree of activity when these $CaCl_2$ solutions are used (table 3). Over-recalcification of the plasma, regardless of its citrate content, prolongs the clotting time and gradually abolishes all evidences of antithromboplastin activity. Under-recalcification also prolongs the clotting time, but greatly accentuates

TABLE 2

Effect on clotting time of a mixture of 0.3 cc. plasma, 0.1 cc. diluted thromboplastin and 0.1 cc. 0.074 M $CaCl_2$, of incubation (5 minutes) of the thromboplastin or plasma in various ways, before recalcification

PLASMA SPEC. NO.	WITHOUT INCUB.	THROMBOPL. AND PLASMA MIXED AND INCUBATED TOGETHER	THROMBOPL. AND PLASMA INCUBATED TOGETHER, NOT MIXED	THROMBOPL. AND PLASMA INCUBATED SEPARATELY	PLASMA ALONE INCUBATED	THROMBOPL. ALONE INCUBATD
	Clotting time in seconds					
1	46	72	53	48	46	46
2	95	139	107	96	92	94

the antithromboplastin effect. Similar trends, though on a larger scale, are observed with hemophilic plasma. In contrast, when the 24 hr. old normal plasma, which had lost most of its antithromboplastin, was tested, the difference between incubated and unincubated specimens was slight. The 24 hr. old hemophilic plasma behaved essentially as a fresh specimen of normal plasma.

The slowing of the rate of prothrombin transformation resulting from an insufficient amount of calcium probably entails a slower utilization of any available thromboplastin. The effect of such a slowing would in turn be magnified by any reduction in potency of the thromboplastin, following incubation with plasma. An exaggeration of the antithromboplastin effect might then be properly expected to occur. Attention has been drawn by Minot and Lee (6) to the relatively greater clot delaying effect of under-recalcification on hemophilic plasma than on normal plasma. The mechanism underlying the elimination of the antithromboplastin effect by addition to the plasma of calcium in excess of that required for optimum coagulation, is not clear.

3. *Platelets and antithromboplastin.* Plasma of different platelet content was obtained by centrifugation of citrated blood for 1, 10 and 45 min., respectively,

at 3,000 RPM. The clotting time of the plain recalcified plasmas and the antithromboplastin effect of each on 2 solutions of thromboplastin were then measured. As shown in table 4, the plasma clotting time becomes shorter and the

TABLE 3

Effect of various amounts of calcium on clotting time and antithromboplastin activity of "cell free" plasma of different citrate content

0.3 cc. plasma, 0.1 cc. thromboplastin (0 minute or 5 minutes incubation), 0.1 cc. $CaCl_2$ solution

TYPE OF PLAS.	CITR. IN PLAS.	INCUBATION	MOLAR CONCENTRATION $CaCl_2$								
			0.027	0.045	0.06	0.074	0.091	0.118	0.14	0.18	0.22
		min.	*Clotting time in seconds*								
Normal, fresh	0.01 M	0	59	53	50	59	73	99	103	167	
		5	126	111	76	78	87	103	100	145	
	0.02 M	0	480	62	56	50	64	89	93	181	
		5	660	103	90	81	88	98	105	117	
Normal 24 hr. old	0.02 M	0	170	52	39	31	34	40	43	48	50
		5	184	63	48	38	34	39	42	42	43
Hemoph., fresh	0.01 M	0	156	122	116	116	143	175	277	348	514
		5	232	181	172	178	179	197	237	275	330
	0.02 M	0	412	121	113	113	123	141	150	198	274
		5	1216	190	182	171	170	184	187	227	237
Hemoph., 24 hr. old	0.02 M	0	439	117	108	104	110	115	126	150	215
		5	626	165	147	132	130	133	136	140	154

TABLE 4

Effect of number of platelets on antithromboplastin activity of a specimen of normal human, citrated plasma

PLASMA SPEC. NO.	NO. OF PLATELETS	CLOT. TIME OF PLASMA + DIL. THROMBOPLASTIN*				CLOT TIME OF PLASMA ALONE†
		Sol. A		Sol. B		
		0 minute incub.	5 minute incub.	0 minute incub.	5 minute incub.	
	thous./c. mm.	*sec.*	*sec.*	*sec.*	*sec.*	*sec.*
1	4	67	103	97	118	390
2	114	72	88	99	104	195
3	420	67	76	96	97	150

* 0.3 cc. plasma, 0.1 cc. dil. thrombopl. sol., 0.1 cc. 0.074 M $CaCl_2$.
† 0.3 cc. plasma, 0.1 cc. 0.074 M $CaCl_2$.

antithromboplastin effect diminishes, as the number of platelets increases. This is especially evident with the weaker solution of thromboplastin. Plasma containing intact platelets is, therefore, unsuitable for these experiments. Although

during the incubation there is some inactivation of the added thromboplastin, upon recalcification fresh thromboplastin is probably released from the platelets, thereby offsetting to a variable extent the inactivating effect of the incubation. This uncertainty as to the amount of thromboplastin derived from disintegrating platelets renders saline or water extracts of washed human platelets likewise unsuitable as a source of this material, instead of dilute brain extracts. Theoretically, platelets should constitute the best source of thromboplastin for this purpose, since the action of antithromboplastin in the circulating blood is probably exerted mainly on the products of platelet disintegration. Nevertheless, suspensions of washed human platelets, alcohol, ether and distilled water extracts of platelets, frozen and thawed distilled water platelet extracts have, so

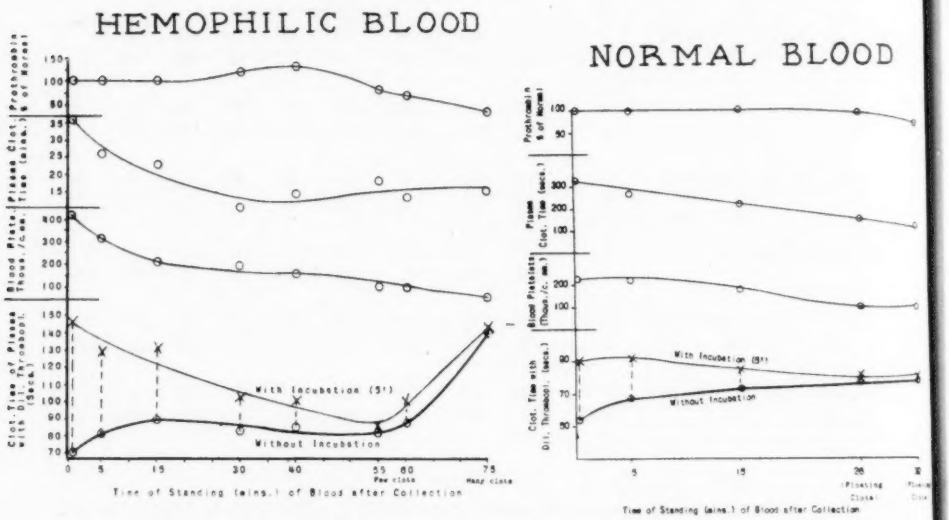

Fig. 2. Changes in prothrombin, platelets, plasma clotting time and antithromboplastin in standing hemophilic and normal blood.

far, in our hands, proved unsuitable as sources of thromboplastin for these experiments. With none of these extracts has it been possible to reduce the coagulation time of 0.3 cc. recalcified citrated plasma to 50 sec. or less.

If plasma is allowed to stand in contact with formed elements of the blood, its antithromboplastin is gradually reduced until no trace of it may be found. A diminution is detected within the first 90 min. of standing (table 5). At the end of the 6 hr. period, when over half the number of platelets has disintegrated, no more activity can be detected. Little change in prothrombin takes place during this interval. As antithromboplastin diminishes and more platelets are destroyed, the plasma clotting time becomes shorter. If the plasma is kept in a paraffin coated tube, the changes may be somewhat delayed. Nearly always, however, no activity may be found after a 24 hr. period of standing. Platelet rich hemophilic plasma may require more than 24 hrs. before all its activity

disappears; platelet free hemophilic plasma may maintain some of it for as long as 8 days. There is likewise a significant diminution in antithromboplastin and in the clotting time of plasma separated from venous blood collected with difficulty or that obtained by an incision of the skin. Transient exposure to tissue juices usually neutralizes all the antithromboplastin in normal plasma, and reduces it somewhat in hemophilic plasma.

The greater coagulability of plasma after standing in contact with formed elements, or with injured tissues must therefore be attributed not only to an increased content of *free* thromboplastin, but also to the diminution of antithromboplastin. These results emphasize the importance of rapidity and thoroughness in collecting the blood and in separating the plasma from its cellular elements, for assurance, before testing the plasma, that there is no cell-bound thromboplastin in it.

4. *Fate of antithromboplastin in shed normal and hemophilic blood.* Twenty five cubic centimeters of normal or hemophilic blood were collected rapidly into

TABLE 5

Changes in number of platelets, clotting time of the plasma, prothrombin time and antithromboplastin in a sample of citrated platelet-rich plasma, standing in a glass tube at 20°C. before being centrifuged and tested

	TIME OF STANDING (MIN.)				
	0	90	180	270	360
No. of platelets (thous./c.mm.)	420	410	340	202	180
Plasma clotting time (sec.)	320	267	235	150	130
Prothrombin time (sec.)	12.4	12.5	12.7	12.5	13.5
Plas. + sol. A + $CaCl_2$					
0 minute incubation (sec.)	65	69	73	77	65
5 minutes incubation (sec.)	95	93	85	82	62

an oiled syringe, without anticoagulants, and allowed to stand in a covered paraffin coated tube at 20°C. At the intervals shown in figure 2, 2.7 cc. of blood were removed from the tube into 0.3 cc. of a 0.129 M sodium citrate solution. After counting the free platelets, the mixture was centrifuged for 45 min. at 3,000 RPM and the plasma examined. Normal blood standing *under the specified conditions* shows little change in the first 5 min. Between 5 and 15 min. there is a diminution in the number of platelets and in antithromboplastin, accompanied by an increase in free thromboplastin as indicated by shortening of the plasma clotting time. For the next 10 min. these changes become even more pronounced, until no significant amount of antithromboplastin can be detected and gross clots begin to appear. The prothrombin, which up to this time had been unchanged, begins to decline, and coincident with it, massive clotting occurs. In a specimen (not shown in the figure) collected at 35 min., and consisting principally of serum, the prothrombin was 7 per cent of normal, the platelets 20,000 per c.mm., the plasma clotting time 440 sec. (thready clot) and there was no trace of antithromboplastin. As a contrast, in hemophilic

blood, only after 55 min. have elapsed and proportionately more platelets have been destroyed, is a pronounced decrease in antithromboplastin observed. Then, clots appear, the prothrombin begins to decline and the plasma clotting time shortens.

It appears, therefore, that in blood shed without contact with tissue juices or water wettable surfaces, actual *coagulation* (formation of thrombin and fibrin) occurs relatively late, though it proceeds rapidly once it has begun. This fact is supported by the knowledge that no significant change occurs in the viscosity of shed blood and no fibrin may be observed in it under the ultramicroscope until after several minutes of standing (7, 8). Most of the time interval between collection of blood and appearance of clots appears to be taken up with the slow release of thromboplastin from disintegrating platelets, and its inactivation by antithromboplastin. After the latter is depleted, any thromboplastin subsequently released from the remaining platelets (or leukocytes) apparently acts directly on prothrombin. These observations make it seem unlikely that free thromboplastin exists, as proposed (13, 10), in the circulating blood. If such were the case the activation of prothrombin should begin as soon as the blood is shed.

5. *Effect of dilution.* Fresh, normal or hemophilic "platelet free" plasma was diluted 1–2, 1–4, etc. with 0.85 per cent NaCl, and the activity of 0.2 cc. of each dilution tested on either sol. A or B by the methods described. The tests must be run *immediately* after each dilution is made, as diluted plasma loses its antithromboplastin potency rapidly on standing. Dilution diminishes the ability of the plasma to reduce the clot accelerating action of thromboplastin (table 6) until a point is reached when the difference between the clotting times of incubated and unincubated plasmas is not materially greater than that between the incubated and unincubated controls (0.85 per cent NaCl and thromboplastin). A test of the thromboplastin inactivating effect of serial dilutions of a given plasma might, therefore, be used as a measure of its antithromboplastin activity. Such activity may be expressed in terms of the smallest amount of plasma which, when incubated with a given volume of a standard solution of thromboplastin, will significantly reduce its clot accelerating action. A significant reduction is considered to exist when such incubation raises the clotting time of the diluted plasma 5 or more sec. above that of a control (thromboplastin solution incubated with 0.85 per cent NaCl). The minimum amount of *normal* plasma required to yield a clear inhibitory effect on 0.1 cc. of sol. A is fairly constant: 0.013 cc., or a 1–15 dilution of normal plasma; only 0.006–0.005 cc. of *hemophilic* plasma (or 1–30 to 1–40 dilution) is needed to produce the same effect. Against the weaker solution of thromboplastin (solution B), greater and more widely divergent amounts of normal plasma are required: 0.1 to 0.033 cc., (1–2, 1–6 dilution) while only 0.006–0.004 cc. of hemophilic plasma are necessary. These findings seem to indicate a clot decelerating activity in hemophilic plasma at least 5 to 8 times greater than normal against thromboplastin solutions producing a clotting time of about 100 sec. In any statement regarding degree of antithromboplastin activity it is important to specify the clotting time of the mixture in which the activity is being tested. While hemophilic plasma main-

TABLE 6

Effect of incubating 0.2 cc. of various dilutions of plasma from 5 subjects (3 hemophilic and 2 normal) with 0.1 cc. of either of 2 thromboplastin solutions, before recalcification and addition of 0.1 cc. of plasma

AMOUNT OF PLASMA IN 0.2 CC.	INCUB. PERIOD	SUBJECT									
		S. M. (hemo.)		N. F. (hemo.)		D. W. (hemo.)		R. B. (Norm.)		J. L. (Norm.)	
		Type of thromboplastin solution									
		A	B	A	B	A	B	A	B	A	B
	min.	*Clotting time in seconds*									
0.2	0	54	98	61	112	69	112	54	114	57	106
	5	93	141	100	159	110	162	76	136	76	126
0.10	0	45	96			63	109		117	53	102
	5	81	140			106	142		128	64	123
0.05	0	47	96						114	54	99
	5	69	134						116	62	120
0.033	0	45						52	111	53	104
	5	67						68	111	62	115
0.02	0	45	106	60	88			48		51	98
	5	65	136	91	117			61		60	104
0.013	0	46	106					48		50	
	5	64	130					56		59	
0.01	0	46	106	62	97	62	109	49		51	
	5	61	128	84	119	83	131	53		51	
0.0065	0	49	110		95	61	110	49		50	
	5	59	128		111	70	126	49		52	
0.005	0	52	109	64	91	58	111	50		51	
	5	60	116	71	106	66	116	48		51	
0.004	0	51	108	65	92	64	112				
	5	55	110	69	103	64	114				
0.0033	0	53	113		91						
	5	55	110		98						
0.85% NaCl	0	53	108	66	90	60	112	47	111	50	102
	5	54	106	65	96	63	115	49	115	51	103
Antithrombopl. Titer* (cc.)		0.005	0.005	0.005	0.004	0.006	0.006	0.013	0.10	0.013	0.033
Plas. clotting time (sec.)		2100		3060		3300		370		354	

* Minimal amount of plasma capable of exerting a significant antithromboplastin effect.

tains, and even increases its activity as the clotting time of the mixture becomes longer (fig. 1) it is not possible to demonstrate this property in normal plasma when the clotting time reaches 130 sec. or over. A convenient point for comparison is that when the 2 unincubated plasmas clot at approximately the same time, with a thromboplastin which is not potent enough to overwhelm the inhibitors. A measure of activity at such levels may, however, apply only to comparisons between hemophilic and normal plasmas. For a comparison of antithromboplastin content of hemophilic plasmas from various subjects, slower acting thromboplastins are desirable.

6. *Effect of heating.* Temperatures of 45° or 50°C. for 5 min. impair somewhat the clot decelerating activity of plasma. Greater impairment follows heating at 55°, and at 65° all activity is destroyed. Antithromboplastin seems to be slightly more heat stable than prothrombin (which is almost entirely destroyed at 55°C.), and not as stable as antithrombin (which resists 60° for 10 min. without much loss in activity). This and the fact that antithromboplastin is almost absent from the blood when thrombin formation begins, makes it seem that the natural antithromboplastin of the plasma is entirely distinct from the natural antithrombin. Another indication of the close specificity of the reaction between thromboplastin and antithromboplastin is obtained by noting the effect of heating the thromboplastin. Temperatures of 100°C. (15 mins.) not only impair its clot accelerating power but almost abolish its susceptibility to antithromboplastin.

7. *Species specificity.* For a clear demonstration of antithromboplastin activity, the brain extracts used as sources of thromboplastin must come from animals of the same species as the plasma being tested. The greatest clot decelerating effect is observed when human thromboplastin is incubated with human plasma; a significant though not as great an effect takes place when rabbit thromboplastin is incubated with human plasma. There is either no significant change or a shortening of the clotting time following incubation of other animal thromboplastins with human plasma. The practice of using heterologous thromboplastins in studies on blood coagulation may be one reason why antithromboplastin activity has not heretofore been observed in human plasma.

8. *Antithromboplastin in plasma globulin fractions.* "Platelet free" citrated plasma separated from hemophilic blood was divided into 3 portions of 10 cc. each: 1, left intact; 2, mixed with 25 $mm.^3$ of concentrated human thromboplastin and incubated for 15 min.; 3, heated to 65°C. for 5 min. and cooled. To each of these portions, 90 cc. of cold distilled water was added, followed by 1.5 cc. of 1 per cent acetic acid, slowly. The cloudy precipitate was packed down by centrifugation and, after removal of the supernatant fluid, dissolved in 5 cc. of 0.85 per cent NaCl and carefully neutralized with dilute NaOH. One sample of 10 cc. of intact normal plasma, collected with the same precautions, was handled likewise. The globulin fraction isolated from hemophilic plasma exerts a clear decelerating effect on the two standard dilute thromboplastin solutions (table 7). The fraction isolated from intact normal plasma has some activity, though less than that obtained from hemophilic plasma. No activity is present in the

fractions separated from hemophilic plasma after preliminary incubation with tissue extract or after heating to 65°C. for 5 min.

One of the explanations for the slow coagulation of hemophilic blood is that its prothrombin is abnormally slow in being transformed into thrombin (2, 9). The validity of this explanation, based on the behavior of prothrombin solutions prepared by dilution and acidification of the plasma has been challenged by Howell (10). Prothrombin, isolated by his acetone precipitation method, behaves alike whether obtained from hemophilic or normal plasma. According to Howell, if dilution and acidification are used, the "plasma thromboplastin" (free thromboplastin) is brought down with the prothrombin; since hemophilic plasma has a low content of free thromboplastin, any prothrombin solution pre-

TABLE 7

Antithromboplastin activity of globulin fractions precipitated by dilution and acidification of variously treated hemophilic and normal plasmas

Figures in the body of the table represent clotting time in seconds

TEST SOLUTION OF THROMBOPL.*	SOURCE OF THE GLOBULIN SOLUTION				CONTROL†
	1. Intact hemoph. plasma	2. Hemoph. plasma + thrombopl.	3. Heated hemoph. plasma	4. Intact normal plasma	
Sol. A					
0 minute incub.	56	46	58	47	43
5 minutes incub.	95	50	63	70	41
Sol. B					
0 minute incub.	84	74	89	87	95
5 minutes incub.	140	82	91	110	92

* Contents of each tube: Without incubation: 0.1 cc. fresh plasma, 0.1 cc. "globulin solution," 0.1 cc. 0.025 M $CaCl_2$, 0.1 cc. thrombopl. sol. A (or B). With incubation: 0.1 cc. "globulin solution," 0.1 cc. thrombopl. sol. A (or B), incubation; 0.1 cc. 0.025 M $CaCl_2$, 0.1 cc. fresh plasma.

† Contents of mixtures as above, except that the globulin solution is replaced by 0.85 per cent NaCl.

pared from it by this method will behave differently from one prepared from normal citrated plasma, which has a higher content of free thromboplastin (10). The present experiments indicate that the difference in behavior of such prothrombin solutions must also be attributed, in part at least, to the greater content of antithromboplastin in the hemophilic plasma fractions.

COMMENT. The foregoing evidence appears to indicate that fresh plasma, *collected with special precautions*, has the property of reducing the clot accelerating action of dilute brain tissue extracts. This property is probably the manifestation of the existence in normal plasma of a substance (or group of substances) endowed with antithromboplastic activity (15). The demonstration of this property is made possible only because addition of sodium citrate to blood immobilizes the prothrombin, thereby preventing its activation by thromboplastin. While prothrombin is not in a reactive state, incubation of plasma with

the thromboplastin solution supplies an opportunity for the inactivating effect of antithromboplastin. When the plasma is recalcified, the prothrombin again becomes reactive; since, however, the potency of the thromboplastin has, meanwhile, been reduced by incubation with the plasma, the conversion of prothrombin to thrombin is correspondingly delayed. Incubation with plasma in which the antithromboplastin had already been exhausted in one way or another, would not be expected to (and does not) affect appreciably the clot accelerating action of the thromboplastin solution. Evidence is now available (4) that in the reaction between thromboplastin and prothrombin some of the thromboplastin is consumed.

When blood, shed without admixture of tissue juices, is allowed to stand "in vitro" a number of platelets (probably less than 10,000 per cmm., or the platelets at or in the immediate vicinity of the blood-glass and blood-air interfaces), swell and disintegrate, releasing a small amount of thromboplastin which is at once inactivated. This process continues until all the antithromboplastin is exhausted, and prevents the free thromboplastin from reaching a sufficiently high concentration to act at once on prothrombin. Whenever and wherever in the plasma, enough free thromboplastin is available beyond that which has been inactivated, it acts on prothrombin and transforms it into thrombin. The eventual excess of the accelerator over its antagonist in normal standing blood, assures a moderately rapid conversion of a large amount of the prothrombin. Since there is an excess of antithromboplastin in hemophilic blood, however, a correspondingly greater amount of free thromboplastin, and a longer time, will be required for this neutralization. As pointed out by several investigators (2, 11, 12) hemophilic blood or plasma can be made to clot within a normal period of time by the addition of an excessive amount of platelets or tissue extract.

Even though, at first sight, the inhibitory effect of antithromboplastin may appear small when compared with the time required for coagulation of hemophilic blood, it must be remembered that in these experiments, this inhibiting property of the plasma is being tested against larger amounts of thromboplastin than are ordinarily released, *at one time*, in blood circulating in the body or standing "in vitro." Moreover, the delay in the inception of clotting of hemophilic blood resulting from an excess of antithromboplastin must also exert, indirectly, a cumulative retarding influence on succeeding or associated changes in the blood (platelet disintegration, prothrombin transformation) and may even help to enhance the effectiveness of antithrombin. Each of these *secondary* effects, namely, slowness of platelet disintegration (6), low content of free thromboplastin in the plasma (10, 13), retarded transformation of prothrombin (9), excess of antithrombin (14), has been proposed, at one time or another, as the primary cause of the coagulation delay. The direct evidence in support of these proposals has, however, not been altogether satisfying. The abnormal stability conferred on hemophilic plasma by its excess of antithromboplastin is probably responsible for the imperfect performance of those functions (15).

SUMMARY

1. In plasma separated from normal human blood, collected with especial precautions, there is a substance (antithromboplastin) which on incubation with dilute extracts of homologous brain tissue, reduces the clot accelerating action of these extracts.

2. Antithromboplastin is exhausted during the stage preceding the inception of clotting; it has a certain degree of species specificity, is made ineffective by dilution, by heating (65°C. for 5 min.), by exposure to tissue juices, or by standing, especially in contact with blood cells.

3. By reducing the amount of available free thromboplastin released from blood or tissue cells, and thereby delaying the activation of prothrombin, antithromboplastin may play an important rôle in maintaining the fluidity of circulating blood and in postponing the inception of clotting of shed blood.

4. Hemophilic plasma has an activity 5 to 8 times greater than that of normal plasma against certain thromboplastin solutions. In standing shed hemophilic blood, more free thromboplastin and a longer time is required for the neutralization of antithromboplastin than in standing normal blood. An excess of antithromboplastin is probably the *primary* cause of the delay in the inception of clotting of hemophilic blood.

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THE EFFECT OF THE SELECTION OF DATA ON THE MEAN BASAL METABOLISM AND THE VARIABILITY OF THE BASAL METABOLISM OF A LARGE SERIES OF COLLEGE WOMEN[1]

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Basal metabolism studies have been made by the workers of the North Central States Co-operative Regional Project as a part of the study of the nutritional status of college women. Early in the study it became necessary to define basal metabolism to provide a uniform method of selection of the data to be used in expressing the results. A review of the literature disclosed that several criteria were used for the expression of the mean basal metabolism.

The diversity of methods used to select data apparently has been due in part to lack of agreement in the interpretation of the concept of basal metabolism. In the minds of some workers, basal metabolism has been the lowest waking metabolism obtainable, though there is nothing implicit in the definition which makes such an interpretation necessary (Means, 1937). More recently the concept of variability in the basal metabolism has developed. Boothby et al. (1936) stated that basal metabolism, as it is measured in the laboratory, is not a lowest or fixed metabolism, but rather a random sample of the metabolism observed under certain arbitrary defined conditions. Rigid adherance to the conditions of measurement will standardize but not fix the response (Boothby et al., 1937). From this point of view the selection of the minimum value to express basal metabolism violates a fundamental principle of the theory of random sampling.

There would be little confusion if each testing laboratory would define their experimental plan and report all data collected. However, detailed comparisons have been attempted between bodies of data which ignore differences which could be due entirely to the selection of the figures employed. In fact, since most experimenters report only that part of their data which they believe pertinent, the material necessary for the evaluation of variability is lost to the reader.

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[5] Approved for publication by the Oklahoma Agricultural Experiment Station, Stillwater.

Berkson and Boothby (1938) presented their studies on variability in basal metabolism. In this publication the variability present in a series of basal metabolism observations was broken down into several parts and the rôle of each defined.

Wishart (1937) has suggested that the metabolism of women, particularly those individuals not living a strictly routine life is especially subject to variation. Rogers (1939) found that a non-student group showed a more uniform basal metabolic rate than the college group and believed it probable that the more settled manner of living of the non-student group might account for this difference.

The study herein reported uses all data except those observations discarded in the laboratory for obvious technical error or non basal condition. An attempt will be made to answer two questions: What effect does the selection of figures have on the derived mean basal metabolism, and, is the character of the sample changed by selection? Standard deviations of the observations on college women by the co-operative laboratories are presented as the first contribution of this type on the variability of the basal metabolism of college women.

EXPERIMENTAL. Data are reported from the laboratories of Iowa State College, Kansas State College, the University of Minnesota, and Oklahoma Agricultural and Mechanical College. Uniform methods were used as defined in the reports of the co-operating group to study the Nutritional Status of College Women (Master Project, 1936 and Minutes of conference meetings, 1936–1940). The subjects were volunteer women students from 17 to 23 years of age. All were judged free from disease known to affect the basal metabolism. It was the intention of the investigators to study a student throughout her four years of college residence. This intention was realized within the limitations of the student's college career. Because of withdrawal from college a certain number were studied for only one year. These individuals have been identified as the "nonrepeat" group. Individuals observed for more than one year have been referred to as the "repeat" group. All academic years have been studied although first year observations only made from 1936 to 1939 are reported in this paper.[6]

The basal metabolism was measured by the Benedict-Roth closed circuit respiratory apparatus with a rubber mouth piece and a nose clip. All equipment was calibrated and checked by accepted means. The subjects came to the laboratory in the morning immediately after rising and with as little expenditure of energy as possible. They rested in bed for at least one-half hour in a room as free from external stimuli as possible prior to making the test. In general, the procedure used was to make two tests on each of two successive or nearly successive mornings, with the exception of the Oklahoma laboratory, where three tests per morning were made. The principles of the test were explained to each subject prior to the first experimental day. Careful records were kept of dates of menstruation and irregularities in the previous twenty-four hour routine. Periods of unusual campus unrest such as examination, etc., were

[6] Because of certain group differences in the variability of the basal metabolism the segregation of students into "repeat" and "nonrepeat" series has been followed in this paper.

avoided. All data were recorded unless at the time of the test and before calculation some error in technic was noticed or the patient was in non-basal condition. From preliminary analyses it was shown that age differences were an unimportant factor in the present analyses.

The data have been segregated by four common methods of selection: first, on the assumption that the six minute observation periods were random samples of the 24 hour basal metabolism, all data regardless of check in clinical percentage

TABLE 1

Mean metabolism and standard deviations based on different methods of selection of data. Nonrepeat data by state

SOURCE OF VARIATION	CALORIES PER SQUARE METER PER HOUR			
	All observations	All check within 5%	Lowest on each day check within 5%	First observation only
Iowa State College				
Mean	34.99	35.00	34.65	35.19
Standard deviation	2.82	2.55	2.59	2.84
Number of individuals	94	53	53	94
5% fiducial limit	34.70–35.27			
1% fiducial limit	34.61–35.36			
Kansas State College				
Mean	34.45	33.75	33.23	34.94
Standard deviation	3.18	2.63	2.64	3.81
Number of individuals	20	12	12	20
5% fiducial limit	33.74–35.15			
1% fiducial limit	33.51–35.39			
Oklahoma Agricultural and Mechanical College				
Mean	33.65	34.15	33.39	34.04
Standard deviation	2.21	1.95	1.91	2.42
Number of individuals	19	9	9	19
5% fiducial limit	33.24–34.05			
1% fiducial limit	33.10–34.19			

were averaged; second, all observations on individuals for whom both the tests within a day and the tests from day to day checked within five percent were averaged; third, the metabolism of a given individual was taken as an average of the lowest values on each of two days which checked within five percent (DuBois, 1930); fourth, only the first observation made was considered (Boothby, Berkson and Dunn, 1936).

The statistical methods and terms used are those of Snedecor (1940) or verbal recommendations of the staff of the Iowa State College Regional Statistical Laboratory. All of the variability measures presented were obtained from data

on the same subjects rather than from the unequal groups as presented by Berkson and Boothby (1938).

RESULTS. Tables 1 and 2 give for each state the means, the standard deviations of the means and the five and one percent fiducial limits obtained by the four methods of selection of data.

TABLE 2

Mean metabolism and standard deviations based on different methods of selection of data. Repeat data by state

SOURCE OF VARIATION	CALORIES PER SQUARE METER PER HOUR			
	All observations	All check within 5%	Lowest on each day check within 5%	First observation only
Iowa State College				
Mean	35.46	35.37	34.97	35.92
Standard deviation	2.39	2.23	2.30	2.56
Number of individuals	48	35	35	48
5% fiducial limit	35.12–35.79			
1% fiducial limit	35.01–35.91			
Kansas State College				
Mean	33.61	33.89	33.54	34.11
Standard deviation	2.74	2.70	2.77	3.17
Number of individuals	22	12	12	22
5% fiducial limit	33.03–34.19			
1% fiducial limit	32.84–34.38			
University of Minnesota				
Mean	35.17	35.08	34.63	35.29
Standard deviation	2.95	2.85	2.83	2.93
Number of individuals	73	42	42	73
5% fiducial limit	34.83–35.51			
1% fiducial limit	34.72–35.62			
Oklahoma Agricultural and Mechanical College				
Mean	34.64	34.20	33.52	35.03
Standard deviation	2.58	2.71	2.75	2.67
Number of individuals	21	11	11	21
5% fiducial limit	34.20–35.08			
1% fiducial limit	34.07–35.22			

The mean obtained by using all the data is very close to that obtained by using only those data which check within five percent. In all cases except the Oklahoma nonrepeat group, the highest mean is obtained when the first observation only was used; the lowest, by method three, from the average of the lowest observations only. Quantitatively, the differences were not great under the

conditions of this experiment in which a series of observations were made on each individual.

For example, in the Iowa nonrepeat group, 34.99 calories per square meter per hour was the mean of the sample. The one percent fiducial limits of the mean of the population from which this sample was drawn were 34.61 to 35.36 calories per square meter per hour. The mean of the observations checking within five per cent (35.00 calories per sq. m. per hr.) fell between these limits. This was true for both the repeat and nonrepeat groups of the Kansas and Minnesota data. The Oklahoma mean for tests which checked within five percent, 34.15 calories per square meter per hour, was just above the upper five per cent fiducial limit, 34.05 calories per square meter per hour but was within the one percent fiducial limit. For the repeat subjects the corresponding Oklahoma mean, 34.20 calories per square meter per hour, fell just on the lower five percent fiducial limit. Apparently these unusual deviations of the Oklahoma data were accidents of sampling since they were not consistent. The majority of evidence tended to indicate that no bias in the mean was introduced by selection of only those data which checked within five per cent.

Selection of the lowest observation on each of two days which checked within five percent gave, in every case, a mean less than that obtained by using all observations. In all cases except the Oklahoma nonrepeat and Kansas repeat series there was less than one percent probability that the mean selected by this method was unbiased. Oklahoma nonrepeat and Kansas repeat series fall within the limits of low probability.

The means based on the first observations on each subject did not give convincing evidence of bias. They were somewhat higher in every case than the means obtained by the use of all data; however, they were not higher than the fiducial limits except in the case of the Iowa repeat group. In the latter instance, the mean obtained by this method, 35.92 calories per square meter per hour, falls just outside of the one per cent probability (35.91).

It will be noticed that the insistence upon a five per cent check very materially reduced the available information, since only from 47 to 73 per cent of the data could be used in any series. However, a more serious criticism was the introduction of bias into the variance in all cases of selection. Since a biased variance invalidates a test of significance, there is no possibility of statistical treatment of such data. If, then, statistical methods are to be used in studying basal metabolism data, the data must include all of the technically accurate observations with no selection after the results have been accepted and calculated.

In table 3 are presented, by state, analyses of variance of basal metabolism using all data obtained.

Certain practical implications may be drawn from the analyses concerning the number of observations necessary to establish the basal metabolism of a given individual. Since the variation within observations on the same day was so small, only one observation per day would seem to be necessary. Since, however, the variance between days of observation on the same individual was highly significant in the case of Iowa and Minnesota, and significant in the cases

of Kansas and Oklahoma, observations on more than one day are necessary to establish the basal metabolic rate of the individual. In the clinic one observation made by an experienced technician should be sufficient to distinguish the pathologic individual. For careful physiological work repeated observations on different days are a necessity. Since little advantage is gained in taking more

TABLE 3

Analysis of variance of basal metabolism using all observations. Nonrepeat and repeat groups by state

SOURCE OF VARIATION	CALORIES PER SQUARE METER PER HOUR			
	Nonrepeat group		Repeat group	
	D.F.	Mean square	D.F.	Mean square
Iowa State College				
Total	375		191	
Individuals	93	24.75**	47	18.02**
Days on individual	94	4.611**	48	2.797**
Within day	188	1.353	96	1.171
Kansas State College				
Total	79		87	
Individuals	19	32.34**	21	23.645**
Days on individual	20	4.538*	22	3.5014
Within day	40	2.315	44	1.7861
Oklahoma Agricultural and Mechanical College				
Total	114		137	
Individuals	18	19.22**	20	34.62**
Days on individual	19	3.354*	21	1.955
Within day	77	1.937	96	1.868
University of Minnesota				
Total			291	
Individuals			72	28.63**
Days on individual			73	3.809**
Within day			146	1.213

* Statistically significant.

** Statistically highly significant.

than one observation per day on an individual, the best proposal for the use of experimental time would be to make a single observation each morning on as many individuals as time permitted. These individuals should be checked on later days.

Table 4 presents a comparison of variability data for the college group with similar data obtained at the Mayo Clinic (Berkson and Boothby, 1938). The

calculations for the college group were made according to Snedecor (1940, chap. 17) and Winsor and Clark (1940). As expected, the inter-individual variability (s_I) was in every instance greater than the intra-individual variability (s_i) so that individuals are distinguished. Except for the relatively small Oklahoma group, the college women presented slightly but consistently higher variabilities than the Mayo Foundation group. Considering the great difference in the sample from which these data were obtained the agreement between this work and that of the Mayo Foundation figures is notable. Though the differences in the size of the standard deviations of the college groups and the Mayo Clinic group are not large enough to warrant any positive assertions, it would seem that college students did show a somewhat more variable basal metabolism which might be associated with the lack of routine in college living.

TABLE 4

Variabilities of basal metabolism of college women expressed as standard deviations in calories per square meter per hour

STANDARD DEVIATION	IOWA		KANSAS		MINNESOTA	OKLAHOMA		MAYO CLINIC*
	Repeat	Non-repeat	Repeat	Non-repeat	Repeat	Repeat	Non-repeat	
s total inter-individual	2.41	2.83	2.77	3.22	2.95	2.23	2.40	2.42
s_I inter-individual	1.95	2.24	2.24	2.64	2.49	2.63	1.77	1.81
Intra-daily intra-individual	1.41	1.73	1.34	1.52	1.10	1.37	1.43	
Day-to-day intra-individual	0.90	1.28	0.93	1.05	1.14	0.31	0.76	0.96
s_i intra-individual	1.08	1.16	1.63	1.85	1.58	1.41	1.62	1.61
Number of individuals	48	94	22	20	73	21	19	

* Berkson and Boothby (1938).

It will be noted that in each school the nonrepeat groups showed a greater variability than did the repeat group. When it is recalled that the nonrepeat group is made up for the most part of individuals who left college before they could be observed a second year, one might engage in interesting speculation, particularly since the number of tests observed for each student was the same and all tests were done in the first year in college.

SUMMARY

A study has been made of the effect which the selection of data exerts on the mean basal metabolism. Of the four methods of selection commonly employed, only one, that of averaging the lowest values on two days which checked within five per cent, gave a mean which was biased. When only the first observation on each subject was used, the mean was slightly higher than that obtained using all the data. It made little difference in mean metabolism whether all data were used or whether a selection was made involving only those data which checked within five percent. Any selection of data resulted in elimination of nearly 50 per cent of the data. However, selective sampling schemes bias the

variance. Bias of the variance invalidates the test of significance and statistical methods then cannot be applied to basal metabolism data which have been subjected to selection.

Analysis of variance indicated that in order to determine the trend of an individual's basal metabolism only one observation per day is needed, but that more than one day of observation should be used.

Standard deviations for total inter-individual, inter-individual, intra-individual, intra-daily intra-individual, and day to day intra-individual variabilities are presented for the basal metabolism of college women.

The variabilities of the repeat groups are somewhat less than the nonrepeat college groups.

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THE EFFECT OF MENARCHE ON BASAL PHYSIOLOGICAL FUNCTIONS IN GIRLS[1]

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Previous investigators have found that physiological precocity, as indicated by early menarche in girls, is usually associated with precocity of growth in body size. Even at the age of 8 or 9 years, girls who begin menstruation at the age of 10 or 11 years, are taller and heavier than girls who do not begin to menstruate till they are 14 or 15 years of age (8, 10, 11). However, few studies of the physiological differences between early and late maturing girls have been made. The following study was planned to throw light on this question and to determine the effects of menarche on basal physiological functions in normal girls

EXPERIMENTAL. Subjects for this study (50 girls) were chosen from the public schools of Oakland, California, solely on the basis of the willingness of their parents to co-operate in a long-term study and the probability of their permanent residence in the community. No physical or physiological characteristic of the child was used as a criterion of selection. The children were first tested when they were between the ages of 11 and 12 years (mean age 11.87 years; S.D. 0.5 year) and were re-tested at six month intervals over a six year period. The following test procedure was followed on each of two successive mornings: The children were brought to the laboratory by automobile before breakfast. Upon arrival, each child lay down on a cot for a 20-minute rest period before measurements were recorded. Systolic blood pressure was read by the auscultatory method at the first sound and diastolic pressures at the fourth sound. The mean of three determinations made at 8-minute intervals was used for tabulation. Pulse rates were counted over half-minute intervals immediate following each blood pressure determination. The average of three observations was used in the tabulations. Basal oxygen consumption was measured over three 8-minute periods by the Tissot open circuit method (3). Siebe-Gorman half-masks were used for collecting the expired air, and expired air was separated from inspired air with Bailey mercury valves (1). The basal respiratory volumes were computed, reduced to standard conditions of 0°C. and 760 mm. Hg. The average values for tests made on the two successive days

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The Oakland Public Schools co-operated in making subjects available for this research.

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were calculated for each subject. These averages were used in the present analysis.

Each month a report was obtained from each girl of the day on which the menstrual flow began and the day on which it ended. In this way accurate dates of first menstruation were obtained. The age of menarche used in this study was the age at the first reported flow, whether regularly maintained thereafter or not. Many irregular periods were reported during the first several months of menstruation in this group of girls (2).

Results. For each girl the time (or age) at which the physiological tests were made was expressed as months before or after menarche, instead of as chronological age. Mean values of blood pressure, pulse rate, basal respiratory volume and basal oxygen consumption were computed for each six month interval before or after menarche.

The results of this analysis are shown in curves plotted as figures 1, 2, 3 and 4. If at menarche there were a sudden change in physiological conditions within the body which produced changes in blood pressure, for instance, curves plotted in the manner outlined above would show a sudden change in slope or direction of trend at the time of menarche. Examination of the curves of figure 1 shows that rising systolic blood pressure in girls occurs prior to menarche and that with the beginning of menstruation, or shortly thereafter, the systolic blood pressure reaches a level beyond which no systematic change occurs. No systematic changes in diastolic blood pressure were observed. The pulse pressure changes (fig. 2) are primarily a reflection of the changes in systolic pressure that occur at menarche.

Figure 2 shows that basal pulse rate rises in girls over the pre-menarcheal period. In the present group of girls the pulse rate began to fall, on the average, 6 to 12 months prior to the beginning of menstruation. The basal pulse rate of girls continues to decrease throughout the remainder of the adolescent period.

At menarche there is a sudden cessation in the increase in basal respiratory volume which has been proceeding throughout the pre-pubertal period (fig. 3). Since total respiratory volume is influenced by body size, basal respiratory volumes were calculated per unit of surface area for each girl. (Surface areas were computed from height and weight by the DuBois (5) formula.) With this correction for the effect of body size, values for basal respiratory volume begin to decrease prior to menarche and decrease more rapidly in the two years following menarche.

Figure 4 shows that prior to menarche the basal oxygen consumption in girls has attained more or less stable values at a relatively high level of consumption. With the beginning of menstruation (or even 6 to 12 mos. before), there is a sudden rapid fall in basal oxygen consumption. Thus the decrease in basal metabolism which characterizes the adolescent period takes place rather rapidly over the two or three years following menarche.

Discussion. The results of this study do not lend support to the view that the beginning of menstruation in girls produces any sudden alteration in physiological conditions which are reflected in pulse rate, blood pressure or basal

metabolism. Although there may be a quickening of the rate of change in oxygen consumption and pulse rate, for instance, after menarche, the curves show that in many individual girls the decrement began 6 to 12 months before

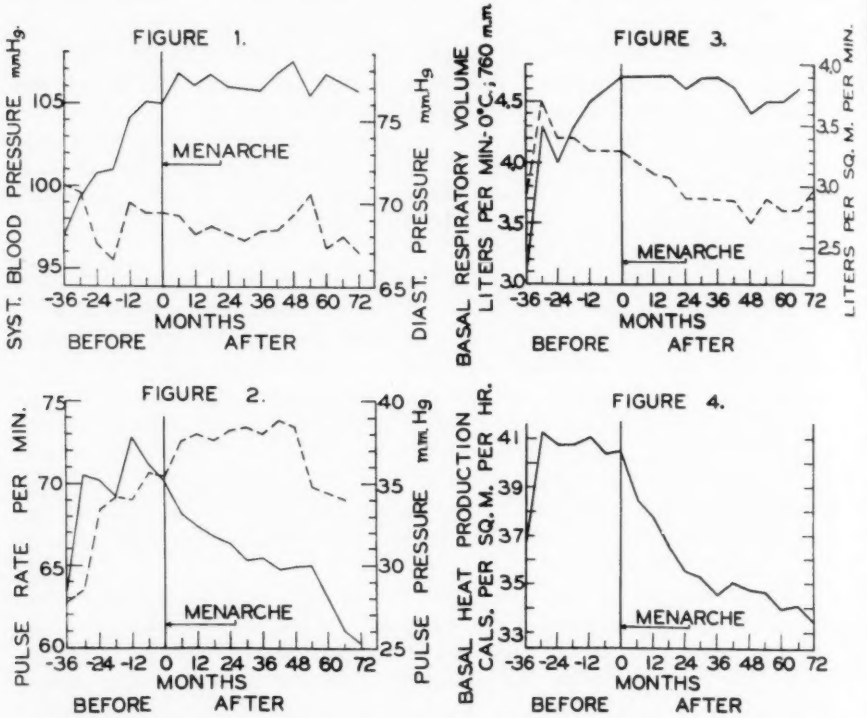

Fig. 1. Effect of menarche on blood pressure in girls. Average values calculated for 6-month intervals before and after menarche for 50 girls. Zero time is taken for each girl at the age when she first menstruated.

——— systolic blood pressure; ---------- diastolic blood pressure.

Fig. 2. Effect of menarche on pulse rate and pulse pressure in girls. See legend of figure 1 for complete description.

——— pulse rate; ---------- pulse pressure.

Fig. 3. Effect of menarche on basal respiratory volume in girls. See legend of figure 1 for complete description.

——— liters per minute; ---------- liters per square meter per minute.

Fig. 4. Effect of menarche on basal heat production in girls. See legend of figure 1 for complete description.

——— basal heat production, calories per square meter per hour.

menstruation occurred. Thus it is safer to regard this period of rapid fall in metabolism as another aspect of maturity which ordinarily occurs about the same time as the first menstruation, rather than the result of endocrine changes associated with menstruation. The changes observed in all the physiological

functions measured are in the direction of adult values and take place at a more rapid rate after menarche than before. Selection of groups of adolescents on the basis of a physiological criterion, such as age at menarche, results in greater homogeneity of the group with respect to other physiological criteria, such as pulse rate, blood pressure or oxygen consumption, than does selection on the basis of chronological age. The periods of rapid change which occur with menarche can be observed in individual growth curves but may be entirely obscured when average curves, based on chronological age, are examined (9). This obscurity is reflected in the disagreement found in the literature on basal metabolism as to the existence of a pre-pubertal rise in basal oxygen consumption (4, 6, 7, 12, 13). The observations of the present study do not begin at an early enough chronological age to answer the question with assurance for girls. However, in many of the girls studied, particularly those who menstruated later, a pre-pubertal rise in metabolism was observed. A rapid fall in oxygen consumption was observed by Johnson (6) in 6 tubercular girls whom he studied continuously over 300 days. Associated with the fall in basal oxygen consumption there was a marked drop in Ca and N retention which continued for 12 to 30 days after menarche.

SUMMARY. Determinations of pulse rate, blood pressure, respiratory volume and heat production under basal conditions were made on each of 50 normal girls at intervals of 6 months between the ages of 11.5 and 17.5 years. All determinations were made under basal conditions on two successive mornings at each six month testing. The Tissot open circuit method was used for estimating basal oxygen consumption during three 8-minute periods on each of the two mornings. Accurate menstrual histories were kept for each subject.

Analysis of curves relating the measured functions to age for each girl shows three marked changes associated with the beginning of menstruation; 1, a leveling off of a pre-menarcheal rise in systolic blood pressure; 2, a decrease in pulse rate, and 3, a precipitous fall in basal heat production per unit of surface area. None of these phenomena are observable in average growth curves based on chronological age because of variations in the age at which onset of menstruation occurs among normal girls.

CONCLUSIONS

1. With the beginning of menstruation there is a rapid decrease in basal oxygen consumption in girls.

2. This period of rapid fall in oxygen consumption is more closely associated with menarche than with any particular chronological age.

3. With the attainment of menarche, other physiological functions, such as blood pressure and pulse rate, rapidly approach mature levels and become stabilized.

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THE MECHANISM OF EXPERIMENTAL RENAL HYPERTENSION IN THE RAT: THE RELATIVE SIGNIFICANCE OF PRESSOR AND ANTI-PRESSOR FACTORS[1]

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The present paper is based on observations made on more than a thousand adult rats in which hypertension had been induced by various operative procedures on the kidneys. The results obtained are in certain respects contradictory to many of the generally accepted concepts concerning the mechanism and treatment of hypertension. They are presented here for their possible value in further elucidating the general problem of experimental and clinical hypertension.

METHODS. Rats have been used exclusively in the present study. Most of the animals were of a piebald strain reared in the laboratory; the remainder, of the Wistar strain, obtained from dealers. The blood pressures were determined by the procedure previously described (1). Hypertension was induced by the application of cloth to the kidney (2, 3).

All operative procedures were carried out under ether and rarely required more than a few minutes for their completion. Attempts were made to minimize trauma, which causes marked drops in the blood pressure of hypertensive animals.

EXPERIMENTAL OBSERVATIONS. *The effect of unilateral nephrectomy.* The failure of unilateral nephrectomy to induce hypertension consistently is well known. However, occasionally hypertension does develop following the removal of one kidney from an apparently normal animal. This is illustrated by the following experiment: The blood pressures of a number of normal rats were determined daily until reasonably constant values were obtained for a period of at least 10 days. The right kidney was then removed under ether anesthesia and the daily blood pressure readings continued. During the week following the operation the general average of the systolic blood pressure of most of the animals was elevated, although only temporarily and to a degree so slight as to be of questionable significance. The subsequent course of the blood pressure varied but, in general, followed one of three courses. In about half of the animals the slight elevation observed post-operatively gradually disappeared and the original pre-operative level was resumed within 10 to 20 days. In most of the other animals, however, the post-operative level remained at a slightly elevated level (5 to 15 mm.). In an occasional animal, on the other hand, the blood

[1] Aided by grants from the John and Mary R. Markle Foundation, The National Life and Accident Insurance and from Mr. Joe Werthan.

pressure remained elevated following unilateral nephrectomy and assumed a definitely hypertensive level of 150 mm. or more which was sustained thereafter.

Although unilateral nephrectomy induces an elevation in blood pressure to a truly hypertensive level in only an occasional rat, the general average pressure in unilaterally nephrectomized animals is definitely higher than in normal animals. For example, in a group of 20 rats, the average systolic blood pressure on repeated determinations was 120, with individual variations of 113 to 126. The average blood pressure level of the same animals during the week following nephrectomy was only slightly altered from the pre-operative level. However, thereafter, the blood pressure as determined at intervals on 20 occasions during the next six months varied between 124 and 138 mm. with a general average of 128 mm. There was thus a definite—although slight—elevation in blood pressure induced by unilateral nephrectomy.

The effect of other unilateral operations on the kidney. The application of silk to one kidney, partial removal of a single kidney and other operative procedures such as applying a partial ligature to the renal artery, all result in effects similar to those described in the preceding section. In many animals these procedures result in a minimal and temporary elevation of blood pressure with a resumption of the normal pre-operative level in the course of two to three weeks. In some otherwise apparently normal animals, operative interference with one kidney (as in the case of unilateral nephrectomy) results in a permanently elevated blood pressure. In others a slight elevation only is induced.

If experimental renal hypertension be due to a liberation of renin, or of an activated renin (angiotonin or hypertensin), it would be expected that the unilateral application of cloth or collodion, for example, or other operative procedure on the kidney, should result in a greater elevation of blood pressure than simple removal of a kidney. In fact, nephrectomy alone could not induce hypertension except by secondary changes which such removal might induce in the remaining kidney. To test this point a group of 20 rats were unilaterally nephrectomized, while in an equal number a collodion capsule was applied to one kidney. The results of this study were as follows:

The average pre-operative blood pressure of the 20 rats was 120, which was identical with that of the group which were unilaterally nephrectomized. The average blood pressures on 15 subsequent determinations from one to six months following the application of collodion to one kidney was 130 mm. as compared to 128 mm. in a similar group of animals which had been unilaterally nephrectomized. This difference of 2 mm. is statistically insignificant in view of the large variations observed in the pressures of the individual animals. However, one point of difference between the two groups may be significant; whereas, only two of the 20 nephrectomized animals died during the period of observation, nine of the group in which collodion was applied to the kidney succumbed between one and six months following operation. This increased mortality may have been due to secondary infection with abscess formation which is not uncommon in animals subjected to the "collodion" operation.

The effect of removing a kidney from rats in which hypertension has been induced

by silk applied to a single kidney or by other operation. The view is widely accepted that experimental interference with the kidneys results in hypertension due to the liberation by the kidney of a pressor substance. If this be true one would anticipate a drop in blood pressure to the pre-operative level following the removal of a kidney to which silk had been applied, or which had been otherwise manipulated to give an elevation of pressure.

The experiments cited in table 1 were carried out to test the above hypothesis. It is evident from these experiments that removal of the presumably injured kidney did not cause the blood pressure to return to the normal level but actually resulted in no change in the blood pressure or in a slight further elevation of the blood pressure.

The effects of bilateral nephrectomy in hypertensive rats. It has long been known that bilateral nephrectomy does not result in the development of hypertension. The explanation for this phenomenon has been that with the removal of all renal

TABLE 1

The effect of removing a kidney to which silk or collodion had been applied with the subsequent development of hypertension on the systolic blood pressure (expressed in millimeters of mercury)

RAT NO.	ORIGINAL PREOPERATIVE BLOOD PRESSURE	AVERAGE BLOOD PRESSURE LEVEL 2 MONTHS AFTER APPLICATION OF SILK TO ONE KIDNEY	POST-OPERATIVE LEVEL			
			1 week	2 weeks	3 weeks	4 weeks
1	120	140	150	155	150	150
2	130	155	150	155	160	160
3	125	150	145	145	155	150
4	130	160	155	160	160	170
5	130	150	160	165	170	170
6	120	145	150	150	150	155
7	115	170	175	175	170	170
8	120	150	150	165	160	160

tissue there remains no possibility for the production of the hypothetical pressor substance responsible for hypertension. To determine if the absence of all renal tissue was compatible with hypertension, one kidney of the rat was removed, and in those cases in which hypertension did not follow, the remaining one was either partially removed or surrounded with silk. When the blood pressure level had reached a constant level for a period of at least 10 days the remaining kidney was freed of adhesions under ether anesthesia and the incisions closed with single ligatures. The purpose of this preliminary operation was to avoid as much trauma as possible when this remaining kidney was removed and thus have the animal in good post-operative state. Otherwise, the effect of shock and uremia would in themselves tend to lower the blood pressure.

The results of these experiments indicated that following the removal of the remaining kidney, the blood pressure did not drop precipitously but tended to gradually assume the shock levels observed pre-mortem in all hypertensive animals, as shown in the experiments cited in table 2.

Comparison of the effects of bilateral nephrectomy, ligation of the ureters and ischemia of the kidneys. Further evidence of the relative effects of deprivation of renal tissue, and the presence of abnormal renal tissue on the blood pressure was obtained by comparing the results of nephrectomy, ligation of the ureter and ischemia of the kidney in animals from which one kidney had been previously removed. This preliminary operation was performed in order to reduce the shock attendant on the bilateral operation. One month after this nephrectomy the blood pressure of the animals was determined daily until relatively constant values were obtained. The animals were then divided into three equal groups. The remaining kidney of one group was removed; the ureter in the second group was ligated and the renal artery of the third group was clamped for thirty minutes. The blood pressures were then determined each morning and afternoon subsequently.

The results of the above described operations indicated that in the rat with complete absence of renal tissue the blood pressure rises in a manner and to an extent equal to that observed in the presence of one kidney the ureter of which

TABLE 2

The blood pressure of hypertensive animals following the removal of their remaining kidney

TYPE OF OPERATION PERFORMED	ORIGINAL BLOOD PRESSURE LEVEL	AVERAGE SYSTOLIC BLOOD PRESSURE LEVELS IN MILLIMETERS OF MERCURY				
		Before final nephrectomy	Following nephrectomy			
			Day 2	Day 3	Day 4	Day 5
Application of silk.....	110	170	170	180	160	100
Application of silk.....	115	180	170	180	160	90
Partial nephrectomy....	100	190	180	160	100	
Partial nephrectomy....	95	200	170	170	120	

had been ligated. Were the rise in blood pressure attributable to the production by the kidney of a pressor substance, one would have anticipated a greater rise in blood pressure in the group of animals in which renal tissue was present.

Ischemia for 30 minutes also resulted in a temporary rise in pressure but this rise was less and of shorter duration than of that observed in the nephrectomized animals or in those in which the ureter was ligated. It is possible that this temporary rise may be due to the liberation from the ischemic kidney of a pressor substance of the nature of angiotonin or hypertensin, but one could equally well explain the observed result as being due to a temporary cessation of renal activity, which in the case of the ischemic kidney is restored after some hours but which is permanent in the case of the nephrectomized animals or those in which the ureter is ligated.

DISCUSSION. Our results are similar in principle but differ somewhat in detail from those recently reported by Friedman, Jarman and Klemperer (4), who also found a failure of the blood pressure to return to normal following re-

moval of the affected kidney. However, these observers reported that unilateral application of cellophane resulted almost uniformly in a steadily increasing blood pressure. Furthermore, pre-operative blood pressure levels of their rats were definitely higher (about 20 mm.) than those which we have observed consistently in our own colony. Whether these differences are to be ascribed to variations in technique, in diet, or in training of the animals is uncertain.

The pathological findings observed by Friedman, Jarman and Klemperer were also different from those which we commonly find following the application of silk unilaterally. If the silk be applied loosely without contraction of the pedicle no superficially observable abnormalities such as were noted by Friedman, Jarman and Klemperer ensue. It is possible, therefore, that the more pronounced tissue destruction induced by the application of cellophane may have caused the liberation of pressor substances not formed when the looser silk is applied. Against this view is the fact that, in our animals, application of collodion unilaterally, which also induces marked destruction of renal cortex, or the ligature of one renal artery also produced only temporary elevation of blood pressure in the majority of animals.

The current views as to the genesis of renal hypertension are 1, that it is due to the production of an abnormal pressor substance by the kidney; 2, that it results from the absence of a normal renal constituent, and 3, that it is due to an imbalance in the ratio of a pressor and depressor substance, both of which are elaborated by the kidney. According to the first view, hypertension is due to renal injury or ischemia resulting in the production of a noxious pressor substance. The second view assumes that the normal kidney has an internal secretory activity which when interfered with results in the hypertension. The last view assumes that renal hypertension is dependent on a disturbed balance between pressor and depressor constituents.

The results of the present study conform most closely to the second of the above-mentioned hypotheses. According to this view the increase in blood pressure which sometimes follows unilateral nephrectomy is due to an incipient deficiency induced by removing functional tissue.

The fact that removal of a kidney to which silk has been applied with the resulting development of hypertension did not cause a return of the blood pressure to normal and the behavior of parabiotic rats (5) also speaks in favor of the "deficiency" theory. Were hypertension due to the production of an abnormal pressor substance or to an imbalance between pressor and depressor or antipressor bodies one would anticipate that removal of such a kidney would cause decline in blood pressure. Such was not the case in these experiments.

It may be argued, of course, that the occurrence of hypertension following removal of one kidney and its failure to be alleviated by removal of a kidney to which silk had been applied or in which hypertension had been otherwise induced, is due to a pressor mechanism set in action by irreversible changes in the second kidney. However, the fact that removal of this second kidney does not cause a reduction in blood pressure as well as the observation that bilateral

nephrectomy causes a measurable (although slight) increase in blood pressure makes this possibility unlikely.

It should be emphasized that the work reported in this communication has been done on rats and that the discussion applies only to experimental renal hypertension as observed in these animals. There is considerable evidence derived from the work of other investigators, that pressor mechanisms may be concerned in the genesis of renal hypertension in the dog. On the basis of the available evidence it does not seem justifiable to conclude either that such mechanisms are active in the rat or that they are absent in the dog. In both species it would appear that deficiency of an anti-pressor mechanism is an important factor in the genesis of experimental renal hypertension.

SUMMARY

Unilateral nephrectomy or the application of cloth or collodion to one kidney caused hypertension in a small percentage of normal rats. The subsequent removal from such animals of the kidney to which cloth or collodion had been applied did not result in a significant decline of the elevated blood pressure. Total nephrectomy in animals surviving sufficiently long tended to cause an elevation of blood pressure. The bearing of these experiments on the theories of the pathogenesis of experimental renal hypertension is discussed.

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CHANGES IN SERUM PHOSPHATE AND CALCIUM AND THEIR RELATION TO MANIFESTATIONS OF TRAUMATIC SHOCK[1]

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During the course of experiments (1) involving the production of shock in dogs by the tourniquet method, the phosphates of the serum were observed to rise as the manifestations of the condition developed. This is reported (2) to occur, also, when shock is induced by repeated bleeding. The study that follows was designed to determine the significance of the increase in serum phosphate.

Effects on infusion of phosphates and their influence on calcium were investigated. The relation of calcium to capillary permeability led to the study of citrates, known to have specific influence on the ionization of calcium. Atropine also was used to aid in the interpretation of the part played by the autonomic nervous system.

Changes in pressure and constituents of the blood and in the electrocardiogram are reported following: I. The release of arterial occluding tourniquets. II. The infusion of phosphates. III. The infusion of citrates. IV. The infusion of citrates in atropinized dogs.

Group I. Changes in pressure and constituents of the blood and in the electrocardiogram following the release of arterial occluding tourniquets on the hind legs of dogs. This group includes 5 animals. They were anesthetized with nembutal, 30 mgm. per kgm., followed by 5–10 mgm. per kgm. when necessary; the tourniquets, applied for 5 hrs., were then released and the femoral artery cannulated for blood pressure determinations. In table 1 are presented changes found in blood pressure, hematocrit, plasma protein, serum calcium, serum phosphate and relative electrical ventricular systole.

With release of the tourniquets, the blood pressure falls and requires $1\frac{1}{2}$ to $2\frac{1}{2}$ hrs. to reach shock level. Other changes include hemoconcentration, loss in plasma protein and marked increase in serum phosphate, accompanied by slight if any change in the total serum calcium. The altered serum phosphate may have a minor tendency to correct itself in the later stages.

Carter and Andrus (3) were the first to describe the prolongation of the electrical systole (Q-T interval) in hypocalcemia.[2] It has been shown (4) that the Q-T interval is in constant relation to the pulse period or the R-R interval. The formula expressing this relationship was found to be $Q\text{-}T = K\sqrt{R\text{-}R}$. This K factor was definitely increased in the two animals for whom it was determined.

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Group II. Changes in pressure and constituents of the blood and in the electrocardiogram following phosphate infusion. Five animals are included in this group. As soon as the nembutal anesthesia was adequate and preparation had been completed for recording the blood pressure in the femoral artery, infusion was begun. Sodium pyrophosphate was used in the majority but, as is indicated

TABLE 1

Changes in blood pressure, blood constituents and electrocardiogram following the release of arterial occluding tourniquets

TIME IN RELATION TO TOURNIQUET PROCEDURE	B.P.	HEMATOCRIT	PLASMA PROTEIN	SERUM Ca	SERUM P	EKG $K = \frac{Q-T}{\sqrt{RR}}$	REMARKS
	mm. Hg	*per cent*	*gm. per cent*	*mgm. per cent*	*mgm. per cent*		
Before application		38.3		9.1	2.3	0.333	Dog 1: sacrificed 3 hrs. after release of tourniquet
Before release	180	40.2		10.1	3.5	0.332	
10 minutes after release	148	47.8		10.1	6.14	0.378	
30 minutes after release	110	48.0		10.0	5.8	0.360	
155 minutes after release	96	45.0		9.0	5.0	0.372	
Before application		42.0	5.22	10.4	3.4	0.324	Dog 2: died 13 hrs. after release of tourniquet
Before release	170	40.0	5.43	11.0	3.28	0.336	
15 minutes after release	126	53.5	5.64	12.1	5.94	0.344	
55 minutes after release	100	55.8	4.90	9.6	5.90	0.320	
160 minutes after release	82	56.8	4.73	8.4	7.94	0.353	
Before application		45.8	5.44		3.34		Dog 3: sacrificed 2½ hrs. after release of tourniquet
Before release	205	51.8	5.34		5.04		
10 minutes after release	146	58.9	5.09		6.60		
40 minutes after release	164	68.3	5.80		7.60		
80 minutes after release	128	65.0	5.28		7.28		
140 minutes after release	80	58.9			6.56		
Before application				9.4	2.28		Dog 4: sacrificed 1½ hrs. after release of tourniquet
Before release	170			9.5	3.54		
12 minutes after release	140			9.7	5.1		
41 minutes after release	138			9.7	6.14		
90 minutes after release	70			9.6	6.34		
Before application		43.7			2.5		Dog 5: sacrificed 2½ hrs. after release of tourniquet
Before release	150	40.9			2.7		
10 minutes after release	126	44.5			6.0		
50 minutes after release	94	52.4			6.0		
120 minutes after release	90	55.2			6.1		

for animal III (table 2), equivalent amounts of sodium phosphate acted similarly. The infusion solution contained 3.3 per cent sodium pyrophosphate in distilled water, adjusted to pH 7.4 with hydrochloric acid. It was introduced at the rate of 1 to 4 cc. per minute, the rate being determined by the general behavior of the animal and particularly by the blood pressure and respiration.

Analysis of table 2 shows a progressive decline of the blood pressure and a marked increase in relative packed red cell volume. Furthermore, the calcium of the serum diminished as the phosphate content increased. This is in agreement with findings of Binger (5). The relative prolongation of the electrical ventricular systole is great as indicated by K values.

TABLE 2

Changes in blood constituents and electrocardiogram accompanying the infusion of sodium pyrophosphate in anesthetized dogs

TIME AFTER START OF INFUSION	B.P.	HEMATOCRIT	PLASMA PROTEIN	SERUM Ca	SERUM P	EKG $K = \frac{Q-T}{\sqrt{RR}}$	TOTAL AMOUNT INFUSED
minutes	*mm. Hg*	*per cent*	*gm. per cent*	*mgm. per cent*	*mgm. per cent*		
0	155					0.359	Dog 1: 4.3 grams sod. Pyroph. [dog died 46 minutes after start of infusion]
22	50					0.438	
37	33					0.417	
0	142	31.3	4.73				Dog 2: 3.63 grams sod. Pyroph. [dog died 107 minutes after start of infusion]
25	98	37.9	4.72				
60	48	45.7	4.94				
100		53.0	5.22				
0		34.8	4.96		3.34		Dog 3: 12.36 grams sod. Phosphate [dog died during following night]
10		39.3	5.06		5.9		
30		40.0	5.27		5.1		
120		54.5	4.32		5.0		
180		44.5					
0	144	38.4		9.6	4.34		Dog 4: 3.3 grams sod. Pyroph. [dog died 40 minutes after start of infusion]
25	80	46.1		3.9	7.16		
0	130	36.0		9.0	2.9	0.326	Dog 5: 3.3 grams sod. Pyroph. [dog died 2 hrs. after termination of infusion]
37	100	39.2		4.4	5.9		
75	70	39.3		3.9	6.6	0.379	
135	67	42.0		3.5	6.7	0 367	
200	42	46.5		4.9	4.5	0.330	

It should be recorded here that tetanic muscular activity made its appearance in all of the animals of this group during the second quarter of the infusion period and became increasingly marked as the serum calcium declined.

Compared with alterations in the same factors after release of tourniquets (group I) the outstanding features in this series are loss of serum calcium, increase in K values and outspoken tetanic muscular activity.

Group III. Changes in pressure and constituents of the blood and in the electrocardiogram following citrate infusion. Five animals are included in this series. The procedure used in the preceding group was followed in detail. The only

variant was substitution of sodium citrate for phosphate. The citrate was a 2 per cent solution in distilled water, adjusted to pH 7.4.

The first striking difference between the effects of citrate and phosphate infusion is the blood pressure response. The gradual progressive and marked fall associated with the phosphate contrasts with only slight change when citrate is used. The pressure falls abruptly only during the last few minutes of the animal's life.

With citrate there is only a moderate increase in packed cell volume, nevertheless the loss in plasma protein is considerable and exceeds what occurs with

TABLE 3

Changes in blood constituents and electrocardiogram accompanying the infusion of sodium citrate in anesthetized dogs

TIME AFTER START OF INFUSION	B.P.	HEMATOCRIT	PLASMA PROTEIN	SERUM Ca	SERUM P	EKG $K = \frac{Q-T}{\sqrt{RR}}$	TOTAL AMOUNTS INFUSED AND REMARKS
minutes	*mm. Hg*	*per cent*	*gm. per cent*	*mgm. per cent*	*mgm. per cent*		
0	115	39.2	4.29	9.9	4.4	0.341	Dog 1: 3.4 grams citrate
18	125	40.3	4.20	10.1	2.0	0.465	[dog died]
56	116	47.1	3.41	10.3	1.4	0.471	
0	90	47.9	5.82	11.3	5.1	0.289	Dog 2: 5.4 grams citrate
16		47.1	5.12	13.4	4.75	0.381	[dog died]
36	75	48.5	5.13	13.6	4.52	0.421	
60		44.2	4.60	13.8	3.52		
80	87	46.1	3.39		3.00	0.362	
0		26.0	5.16	10.8	2.96	0.339	Dog 3: 3.6 grams citrate
20		25.9	4.59	11.5	4.00	0.476	[dog died]
48		30.5	4.53	11.5	3.68	0.366	
0	98	36.5	6.13				Dog 4: 4.8 grams citrate
38	105	35	5.42				[dog died]
0	160	38.7	6.26				Dog 5: 5.6 grams citrate
40	126	39.8	5.47				[dog died]
68	108	44.6	4.64				

phosphate though this is associated with much greater increase in relative packed red cell volume. The serum calcium actually tends to rise as opposed to its fall with phosphate but the tetanic state associated with the two substances cannot be differentiated and the relative electrical ventricular systole is prolonged with citrate as it is with phosphate even though the total serum calcium certainly is not reduced with the former (6).

These facts question the accepted theory concerning the relation of the relative electrical ventricular systole, as indicated by the K factor, to the total calcium of the serum. They suggest that the K factor may be related to a particular calcium fraction. That this may be ionized calcium is indicated

by the fact that the tetanic, muscular contractions manifested equally after citrate or phosphate infusion are relieved promptly in both circumstances by injection of small amounts of ionized calcium salts.

Group IV. Changes in pressure and constituents of the blood and in the electrocardiogram following citrate infusion in atropinized dogs. Five animals comprise this series. The procedures were identical to those for group III with the single exception that the animals were given 0.3–0.5 mgm. per kgm. of atropine sulphate intravenously five minutes before the beginning of the infusion of citrate.

The outstanding difference between the atropinized and non-atropinized dog is the amount of citrate tolerated. With the latter, death invariably terminated the experiment in about 1 hour when 3.4 to 5.4 grams of citrate had been infused. After atropine more than twice this amount of citrate was infused, with tetanic muscular activity as the only untoward symptom.

Naturally more than twice the time for the larger quantity of citrate was required as all of the infusions were made at approximately the same rate. During this longer period for the atropinized animals the blood pressure was sustained at the pre-injection level or even higher and all five dogs were in fair condition when the infusion was discontinued.

Dog 2 received 14 grams of citrate and died after an hour; dog 4 lived 4 hrs.; the other three (1, 3 and 5) recovered completely from the experimental procedures. The tetanic muscular contractions could be stopped with calcium chloride, and the alterations in the K values and changes in the blood constituents including serum calcium and phosphate, conform to those of group III. There is some hemodilution after atropine itself, but only slight variation in relative packed red cell volume occurs during the remainder of the procedure. The plasma protein loss, an outstanding feature with the non-atropinized dog, is materially reduced, but is still noteworthy. It bears no constant relation to fluid loss from plasma. All of these factors are included in the table 4.

Discussion. The increase in serum phosphate following the release of tourniquets applied for 5 hrs. to the hind legs of dogs raises the question concerning its source. The fact that there is also an elevation of serum potassium (7) is supporting evidence that these electrolytes come from tissue cells distal to the tourniquets that are damaged during the period of ligation.

When cells are so altered that they lose salts like potassium and phosphate other cellular functions also may be modified with the result that altered metabolic products are liberated and, with release of the tourniquets, absorbed in sufficient quantity to participate in the production of shock. This hypothesis is by no means new but adequate support has not been forthcoming (8).

The augmented serum phosphate may be an expression both of a major discharge of this electrolyte into the circulation and a coincident depression of the ability of the kidneys to eliminate it. The latter condition is associated with the slowed circulation. An additional reason for the reduced excretion may be the nembutal anesthesia.

The rise in serum phosphate from 2 mgm. per cent to as much as 7.6 mgm.

per cent after the release of the tourniquets is only relatively important, if the following premises are correct. The effects of phosphate are associated primarily with its influence on calcium. As has been shown, the total serum calcium is practically unaffected and the ionized calcium, as far as this may be evaluated

TABLE 4

Changes in blood constituents and electrocardiogram accompanying the infusion of sodium citrate in anesthetized atropinized dogs

TIME AFTER START OF INFUSION	B.P.	HEMATOCRIT	PLASMA PROTEIN	SERUM Ca	SERUM P	EKG $K = \frac{Q-T}{\sqrt{RR}}$	TOTAL AMOUNTS INFUSED
minutes	*mm. Hg*	*per cent*	*gm. per cent*	*mgm. per cent*	*mgm. per cent*		
0		28.2	5.51	10.2	3.2	0.283	Dog 1: 10 grams citrate
30		28.9	4.59	11.2	2.4	0.359	[dog survived]
60		37.6	4.81	10.1	2.0	0.359	
90		38.4	4.50	9.3	1.5	0.347	
150		40.1	5.09	9.6	1.4	0.347	
0*	66	36.8	5.25	11.0		0.344	Dog 2: 14 grams citrate
0	66	28.1	4.73	10.5		0.348	[dog died 1 hr. later]
54	60	28.9	4.29	12.0		0.389	
102	80	30.1	4.04	14.0		0.422	
147	80	32.0	4.01	12.5		0.398	
0*	142	41.9	5.13	10.4	4.0		Dog 3: 10.5 grams citrate
0	142	33.8	5.05	10.4	4.2		[dog survived]
42	135	33.0		13.1	4.2		
85	108	40.0	4.32	13.1	4.4		
140	140	40.0	3.85	11.4	4.4		
0*		43.8	5.16	10.7	3.3		Dog 4: 7 grams citrate [dog
0		33.6	4.97	10.8	3.6		died 4 hrs. later]
43		41.0	5.03	10.8	2.7		
91		43.8	4.85	10.1	1.9		
138		44.8	4.77	10.0	1.95		
0*		38.7	5.18	11.2	2.2		Dog 5: 7.2 grams citrate
0		32.0	5.25	10.7	2.4		[dog survived]
48		32.0	5.10	10.8	2.7		
106		39.3	5.23	10.8	2.1		
155		38.4	4.46	7.5	3.1		

* Before atropine.

from the length of the electrical ventricular systole, as expressed by "K" values is only slightly changed.

Equal elevation of serum phosphate cannot be attained by phosphate infusion in the unanesthetized dog for reasons already given. With nembutal narcosis, on the other hand, serum phosphate piles up on infusion and is accompanied by a corresponding fall in serum calcium with manifest tetanic muscular activity

and prolongation of the relative electrical ventricular systole. When citrate is infused the serum calcium behaves quite differently. Its total quantity does not fall, indeed it may be increased to 12 or even 14 mgm. per cent. Even so, the same tetanic muscular contractions occur and the prolongation of the relative electrical ventricular systole may be greater. These muscular and cardiac dysfunctions can be corrected promptly by the injection of small quantities of ionized calcium.

Differences in the loss of fluid and of protein through the capillary wall and the changes in the fractions of serum calcium included in this series of experiments are of interest in association with mechanisms concerned in capillary permeability. Phosphate infusion results in considerable loss of fluid from the plasma; when citrate is used the loss in fluid is small, in protein great. This provides further evidence that particular conditions determine the permeability of the capillary wall for different constituents of the plasma.

The decrease in total serum calcium and the concomitant hemoconcentration with phosphate infusion together with the increase in total calcium without loss of fluid from the plasma with citrate, support the theory that calcium is the determining factor for the passage of fluid through the intercellular "cement" substance of the capillary wall (9). The theory is weakened through the fact that the total calcium is not changed materially when shock is produced by the tourniquet method and the loss of fluid nevertheless is considerable.

Even if the interaction of calcium and cement substance was determining for the passage of fluid, this would not hold for protein. Its loss is maximal with citrate infusion when the total calcium is not reduced and indeed augmented. That calcium made dialysable by citrate (6) may be washed away from the cement substance during the citrate infusion to allow the passage of protein is incompatible with the fact that there is no associated hemoconcentration.

Available knowledge does not exclude passage of fluid through the cement substance; it does support the assumption that this is not likely for protein and that the capillary lining cells are involved. The hypothesis is strengthened by the fact that the loss of protein through the capillary wall with citrate is materially reduced by atropinization before infusion. Under these circumstances it would be more probable that neural control involves the lining cells of the capillary rather than the cement substance.

It can only be queried whether atropinization that allows survival after what would otherwise be more than twice the fatal quantity of citrate is dependent upon the known action of the drug as a definite coronary vasodilator (10).

SUMMARY

1. A rise in serum phosphate accompanies shock produced by tourniquets on the hind legs of dogs. No large changes occur in the total serum calcium or in the "K" values of the electrocardiogram.

2. Infusion of phosphate in the anesthetized animal is followed by increase in serum phosphate, loss of total serum calcium, fall in blood pressure, hemo-

concentration, tetanic muscular contractions, prolongation of the relative electrical ventricular systole (K value) and death.

3. Infusion of citrate in the anesthetized animal does not influence blood pressure until the terminal moments of life; only moderate hemoconcentration occurs but there is marked loss of plasma protein. The total serum calcium tends to rise even though tetanic muscular contractions and alteration of the electrical ventricular systole are outspoken.

4. The atropinized animal survives the infusion of more than twice the amount of citrate which is fatal without the use of this drug. The only other difference manifested is a decrease in the loss of plasma protein.

5. These findings provide a basis for consideration of the mechanisms involved in permeability of the capillary wall.

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THE TOXICITY OF THORACIC DUCT FLUID AFTER RELEASE OF TOURNIQUETS APPLIED TO THE HIND LEGS OF DOGS FOR THE PRODUCTION OF SHOCK[1]

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This study was designed to explore the effects of injection of thoracic duct fluid, collected promptly after the release of tourniquets applied to the hind legs of dogs. Some of the premises for the undertaking include the marked rise in serum phosphate (1) and potassium (2) found in the same circumstances. On the basis that these electrolytes are derived from the cellular elements of the legs distal to the tourniquets and that they are evidence of disintegration, it was thought likely that other materials, including protein derivatives or degradation products, also might be released and be absorbed from the tissues through the lymph.

Study of the blood from such extremities has not been rewarding (3) and results concerning the effects of extracts of the tissues of the leg are conflicting (4). On the other hand, it has been shown that taping of the legs before or immediately after release of the tourniquets reduces the mortality (5). Such taping may prevent loss of fluid into the damaged tissues at the site of application and distal to the tourniquets and also absorption of toxic substances through the lymphatics. Freezing of the legs during the period of ligation has similar effects (6).

The first results of this study were not convincing. They became more satisfactory when it was realized that blood pressure changes and other major evidences of shock, occasionally leading to death, might follow nothing more than nembutal anesthesia.

Following the description of methods several series of experiments will be reported. These indicate differences in the reactions to nembutal as compared to thoracic duct fluid and also differences in the reactions to such fluids from dogs with and without preliminary use of tourniquets.

PROCEDURES. Shock was produced in dogs, after the administration of nembutal, by the application of tourniquets as high as possible on both hind legs. The tourniquets were applied not only to occlude the circulation of the extremities but tightly enough to bruise the underlying soft tissues. After five hours the thoracic duct was exposed as follows:

The median border of the left jugular vein was brought into view by an incision extending upward from the sternal notch along the edge of the left sternocleidomastoid muscle. The vein was followed to its juncture with the left subclavian. When the latter was elevated the thoracic duct became visible

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as did the larger tributaries, usually two, that join to form a sac emptying posteriorly into the vein. Constriction of this vein on the cardiac side of the entrance of the lymph sac caused the ducts to become engorged so that they could be differentiated readily by their color from veins. After ligation of the ducts from the left front leg and from the head, the thoracic duct was incised a centimeter below the lymph sac to avoid valves that might complicate canalization.

The duct collapses as soon as it is cut and the opening, made preferably with small sharp scissors, should be kept in sight constantly until the cannula (a glass tube with a bore of 1.5 mm.) is fixed in place. It is important not to use force for insertion of the cannula. Lymph usually begins to well out of the free end of the cannula promptly. When this does not occur suction should be avoided as it will collapse the thin-walled duct. Introduction of 0.5 cc. of air through the mouth of the cannula is helpful in causing a free flow. The lymph from a normal dog, not fed for 12 hrs., usually is opalescent, rarely milky; it becomes pink and at times frankly blood-stained within a few minutes after release of tourniquets applied to the extremities.

The lymph was collected from the cannula in a heparin-containing tube immersed in a salt-ice mixture. Its rate of flow varied and was more rapid in young animals. Usually up to 20 cc. were collected in from $\frac{1}{2}$ to 2 hrs. The experiments will be presented in two major groups differing from each other only in the amount of nembutal used for narcosis. Each of these major groups, to be designated as I and II, includes two series, one involving the use of thoracic duct fluid from normal dogs (A), the other thoracic duct fluid collected after release of tourniquets applied to the hind legs (B).

Group I. The animals included in this group received an initial intravenous injection of 30 mgm. of nembutal per kgm. body weight; when necessary 10 mgm. per kgm. body weight were given later in the experiment.

(A) *Normal thoracic duct fluid.* This series includes 28 dogs. Fourteen of these supplied the thoracic duct fluid for injection into the other fourteen. In each instance the fluid for injection into an animal was derived from a single donor.

The recipients were small in contrast to the donors, which were selected for their large size. Like the donors they were anesthetized with 30 mgm. of nembutal per kgm. body weight. After the femoral blood pressure record was started, from 7 to 20 cc. of thoracic duct fluid were injected into a vein of the foreleg. The observations were discontinued if no change in the animal's blood pressure or general condition occurred within two hours. If a change occurred the records were continued until the blood pressure reached the pre-injection level.

All of the animals survived the procedure and subsequently developed no untoward signs. Half of them showed no change in blood pressure. The other half had a fall in pressure, which was gradual, varying in onset from 10 to 70 min. after injection of lymph; lasting from 40 to 300 min. and representing a drop from 30 to 70 mm. Hg. The time lapse between injection and beginning

of the fall in pressure, its duration and extent do not bear any relation to each other or to the amount of lymph injected. These facts are included in table 1.

(B) *Thoracic duct fluid from animals in shock.* This series includes 36 animals divided equally into donors and normal recipients. Twelve of the latter, 66 per cent, showed a fall in blood pressure as compared to 50 per cent in series "A". The fall was gradual, varying in onset from 10 to 60 min. after the injection of lymph, lasting from 45 to 240 min. and representing a drop of from 45 to 100 mm. Hg. Three of the animals died within a few hours. The fall in blood pressure showed only a slight rise before death. The other nine dogs recovered. The amount of lymph varied from 7 cc. to 51 cc., and it is interesting that the animal receiving the smaller dose was one of the three which died. The results are summarized in table 2.

Group II differs from the one just presented in: 1. The amount of nembutal used for narcosis. 2. The manner of its administration. 3. Preliminary ad-

TABLE 1

Effect of lymph from normal dogs (30 mgm. of nembutal per kgm. body weight)

DOG	BLOOD PRESSURE BEFORE INJECTION	ONSET OF FALL (TIME AFTER INJECTION)	DURATION	EXTENT	AMOUNT OF LYMPH INJECTED
	mm. Hg	*min.*	*min.*	*mm. Hg*	*cc.*
1–7		none	0	0	10, 16, 12, 11, 16, 14, 20 respectively
8	150	10	120	50	12
9	105	15	240	60	17
10	70	20	120	30	11
11	110	40	300	60	18
12	100	45	40	45	10
13	120	65	210	70	9
14	130	70	120	50	8

ministration of the anesthetic several days prior to the experiment with recording of blood pressure.

These precautions were deemed essential as it was found that a fall in blood pressure may occur with the larger dose of nembutal even though no other procedure is involved. Occasionally an animal may even die.

The initial quantity of nembutal given to all of the animals included in group II, both donors and recipients, was 25 mgm. as distinct from 30 mgm. per kgm. body weight for group I. When subsequent injection of the drug was necessary on account of the length of an experiment 2 mgm. were used as distinct from 10 mgm. per kgm. body weight for group I. Care was exercised to inject the nembutal saline solution (10 mgm. per cc.) slowly and uniformly over a period of 2 min.

The reactions of many of the animals in both series "A" and "B" to this amount of nembutal were determined days before the final experiment. In no instance was there any fall in blood pressure, with the lighter anesthesia. There

is, in fact, a tendency to a slight rise in pressure. This control reaction is recorded for the animals in which it was used.

TABLE 2

Effect of lymph of dogs in shock (30 mgm. of nembutal per kgm. body weight)

DOG NO.	BLOOD PRESSURE BEFORE INJECTION	ONSET OF FALL	DURATION	EXTENT	AMOUNT OF LYMPH INJECTED
	mm. Hg	*min.*	*min.*	*mm. grad.*	*cc.*
1–6		none	0	0	9, 17, 7, 10, 9, 18 respectively
7	115	10	45	40	15
8	90	10	120	50	13
9	90	10	40	50	12
10	110	13	45	40	13
11	120	15	120	55	9
12	130	20	120	90*	12
13	130	30	75	85	7
14	130	30	160	90	11
15	95	30	120	50	10
16	140	40	240	100	9
17	110	55	120	55	7
18	115	60	120	35	15

* Died.

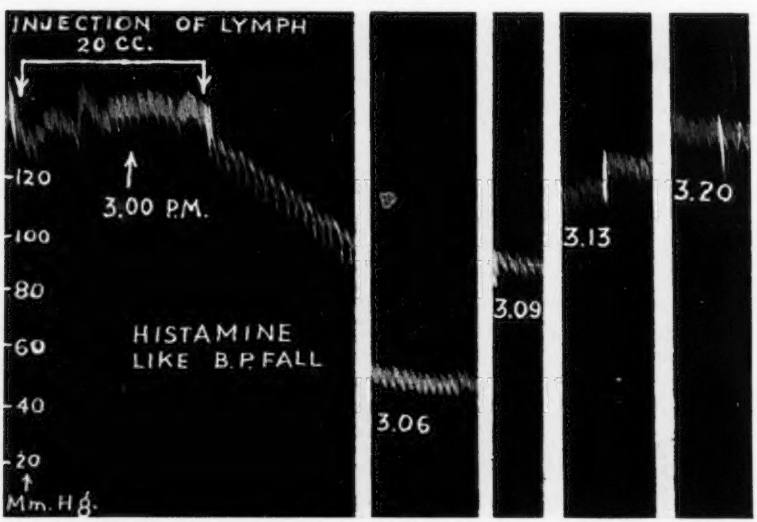

Fig. 1. Fall of blood pressure following the injection of lymph from a dog in shock. The curve resembles that after the injection of small doses of histamine.

A. *Normal thoracic duct fluid.* The series includes 20 animals divided equally into donors and recipients.

The experiment involved the intravenous injection of thoracic duct fluid from a separate donor for each recipient, as described for the previous series. Only

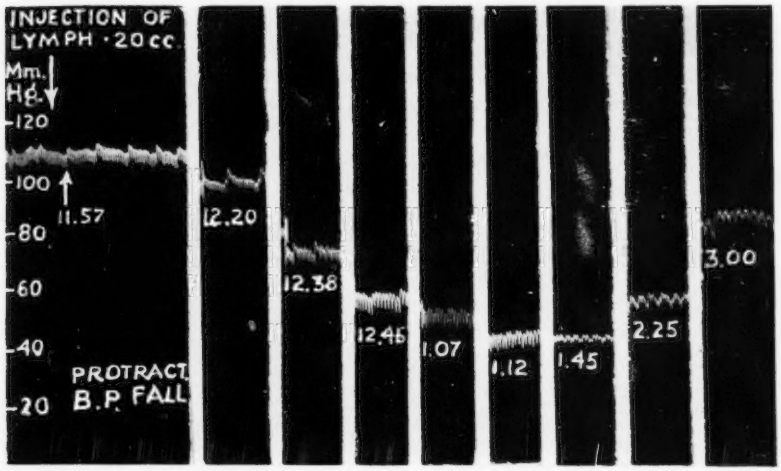

Fig. 2. Fall of blood pressure following the injection of lymph from a dog in shock. In contrast to figure 1 the onset is delayed, the depression is gradual and the blood pressure has not quite returned to its original level after 155 min.

TABLE 3

Effect of lymph of dogs in shock (25 mgm. of nembutal)

DOG NO.	BLOOD PRESSURE BEFORE INJECTION	ONSET OF FALL	DURATION	EXTENT	AMOUNT OF LYMPH INJECTED
	mm. Hg	*minutes*	*minutes*	*mm. Hg*	*cc.*
1–8*		0	0	0	25, 19, 13, 20, 12, 16, 20, 7 respectively
9*	145	Immediate	6†	90	7
10*	145	Immediate	8†	80	28
11	130	Immediate	8†	80	20
12*	140	1	36‡	30	15
13	140	2	13†	90	20
14	120	3	180‡	120	13
15*	155	5	120‡	80	15
16*	140	6	40‡	90	14
17	105	16	90‡	26	16
18	105	25	155‡	58	20

* Preliminary determination of the effect of nembutal.
† Sharp drop.
‡ Gradual drop.

one of the recipients showed any change in blood pressure. A fall began 1 min. after the injection of 20 cc. of lymph, gradually reached its maximum of 35

mm. Hg, and returned to the pre-injection level all within the next 3 min. Three of the other nine animals that showed no reaction also received 20 cc. of lymph.

B. *Thoracic duct fluid from animals in shock.* This series is made up of 36 dogs divided equally between donors and recipients. Of the latter, ten were tested some days previously for their reaction to the smaller dose of nembutal. They showed no change in blood pressure and no demonstrable general effect either during the narcosis or in the following days prior to the experimental procedure.

Nine of the 18 recipients showed a fall in blood pressure. In four this resembled the response to the injection of small amounts of histamine.

Figure 1 represents the record of one of these dogs. The fall was fairly sharp and promptly followed the injection of the thoracic duct fluid; its duration varied from 6 to 13 min. and its extent from 86 to 96 mm. Hg.

The response of the other 5 animals was represented by a smooth curve. The fall of pressure began less promptly, 1 to 25 min. after the injection of thoracic duct fluid and continued for a considerably longer period, from 36 to 120 min., in the animals that recovered (fig. 2).

This sequence of events occurred in all 5 animals; in one, however, the pressure failed to rise again and the animal died. The amount of lymph used for the individual dog varied from 7 to 25 cc. As can be seen from the table that follows, the dose of lymph is not related to the type of curve or the extent of the fall in pressure (table 3).

SUMMARY

The thoracic duct fluid becomes blood-stained after the release of tourniquets applied to the hind legs of dogs. When this fluid is injected intravenously its effect upon blood pressure is variable. Injection of as little as 7 cc. may be associated with a severe and protracted fall in blood pressure which may terminate in the death of the animal.

The vasodepressor effect is more frequent when large doses of nembutal have been used for narcosis.

Injection of such amounts of nembutal may be associated with a fall in blood pressure even when no other procedure is involved.

When narcosis is controlled to avoid vasodepression injection of thoracic duct fluid from normal dogs has no significant effect.

In contrast, a fall in blood pressure follows in 50 per cent of the animals injected with the thoracic duct fluid of animals shocked by the tourniquet method.[2]

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[2] These findings are in accord with those of Doctor A. Blalock (personal communication).

STUDIES ON THERAPY IN TRAUMATIC SHOCK[1]

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The approach to the study of therapy in shock selected for this investigation was the well known application of tourniquets to the hind legs of dogs (1, 2, 3). This method of inducing characteristic manifestations of the condition was chosen on the basis of experience with the procedure modified, as will be seen, to provide a standard that yielded a satisfactorily uniform clinical picture as a measure of therapy.

The therapy was devised to determine the relative importance of well recognized factors like the circulating blood volume (4) as this is affected by the content of the capillary bed, capillary permeability, etc. Particular attention was directed toward the effect of plasma infusion as compared to plasma-albumin solutions with similar colloidal osmotic pressure. In addition, methylene blue and succinate were used in order to compare their known action on tissue metabolism with the effect of solutions exerting a high colloidal osmotic pressure.

Without doubt there are many stages in the development of shock that may rectify themselves or be corrected by agents designed to compensate for manifestations of inadequate circulation. Long experience with various substances—culminating with serum albumin—allows no other conclusion.

These positive results, however, leave the question open concerning the stages of shock in which such therapy is not adequate. Then queries arise concerning the extent to which impaired circulation may be responsible for metabolic changes like inhibition of cell respiration, alteration of glycolysis or glyconeogenesis. Such deficiency may follow other and more direct cause than impaired circulation and be the basis for the theory of the toxic genesis of traumatic shock.

The presentation that follows deals first with the problems underlying the adoption of the particular procedure utilized for the production of shock and then with a series of therapies designed to ascertain the relative importance of the mechanical circulatory disturbances as distinct from those associated with changed metabolism.

PROCEDURE. The method used in this study for the production of shock and its therapy was developed with particular consideration of the influence of various factors known to affect the responses of animals. They include nembutal anesthesia, repeated withdrawal of blood for analyses during the course of the individual experiment, the application of the tourniquets to the legs with particular reference to the amount of underlying muscle and the traumatization effected through the pressure exerted by the tourniquet and finally the local

[1] Aided by grants from the Commonwealth Fund and the Markle Foundation.

swelling in the traumatized area associated with local edema. Each of these problems will be presented now.

It is well known that the anesthetic nembutal also exerts profound side effects,—the respiratory rate is markedly reduced, the blood pressure falls, there is hemoconcentration with loss of protein as well as fluid from the blood, the renal function is suppressed and the body temperature falls. The picture in its totality is difficult to differentiate from shock produced in other ways; it may actually terminate with the death of the animal. While there are undoubtedly individual variations in the response to nembutal on the part of animals, the major factors are the size of the dose and the method of its administration.

A dose of 30 mgm. per kgm. introduced intravenously and slowly is tolerated without serious manifestation by the majority, but one out of four animals will develop more or less serious signs of the variety enumerated above that would confuse the interpretation of further procedure in the production of shock. This is avoided when 25 mgm. is the dose.

A complicating factor is the long period of anesthesia required by the tourniquet method of producing shock. Even 30 mgm. per kgm. usually requires reinjection of smaller doses after several hours. The amount of the drug used for reinjection must be small to avoid untoward signs: 10 mgm. per kgm. is an excess, 5 is adequate and this often may be reduced to 2 mgm. per kgm. The procedure adopted therefore consists of an initial dose of 25 mgm. per kgm. followed as required after several hours by reinjection usually of 2 mgm. per kgm. even though at times this has to be repeated.

The smaller initial and repeat doses of nembutal were selected because the blood pressure tended to rise in contrast to the tendency to fall with the larger doses. The blood pressure proved to be the most satisfactory indicator of the general condition of the animal as influenced by nembutal.

Another reason for the selection of the quantity of nembutal is the fact that the smaller doses exert less influence than the larger amount on the circulating blood volume and any tendency to a reduction in this volume must be avoided. This is evidenced since even the withdrawal of blood for essential analyses in control experiments facilitated the development of shock. As would be expected, the more blood withdrawn from the circulation, the greater the effect. With this in mind, no blood was withdrawn in this study as the objective was the development of shock by the use of the tourniquet method, and its therapy, with elimination, so far as possible, of contributory influences.

As soon as anesthesia was evident the tourniquets were applied to the back legs. Care was taken in the selection of the rubber tubing, the place of application and the tension exerted. Pure gum hand made tubing was used with a bore of $\frac{5}{16}$ inch and a wall of $\frac{1}{16}$ inch with maximal elasticity and tensile strength indicated by the fact that it was able to suspend a 75-pound weight. It was applied as high as possible on the thigh and fully extended so that it would exert a maximal pressure on the underlying musculature. This was found to be necessary as mere occlusion of the circulation is not followed by shock in a sufficiently high and constant percentage of animals.

When the tourniquet is applied not only to occlude the circulation but to exert a maximal pressure on the soft tissues of the thigh as manifested by hemorrhage in the muscle and even in the skin adjoining the groove made by the rubber tube, the release of the tourniquet is followed promptly and constantly by brawny induration of the leg. This begins as a rule in the inner thigh, extends circularly and distally and attains its height in one hour. By this time the blood pressure has dropped markedly and is approaching the shock level, providing, with sufficient speed and constancy, opportunity for the study of therapy.

As has been pointed out, the method of applying the tourniquet is a factor in the swelling of the extremity after the pressure is released. With only vascular occlusion there is little edema of the leg; with traumatization of the soft tissues the edema is marked. As will be seen even the more extensive swelling of the leg that occurs when the tourniquet has been applied as tightly as possible is greatly increased if the animal lives for a few hours and has had the circulating fluid of its blood replenished with saline or glucose. To avoid this complication as a factor in the outcome, the legs were firmly taped as high as the ilio-femoral joint and including the buttocks, thus limiting further local loss of fluid. The right leg was taped one hour after the release of the tourniquet at a time when, as has been said, the pressure had approached the shock level; the left was taped one hour later. This delay was necessitated by the fact that the left femoral artery was used to make the kymograph records as it was deemed desirable to avoid structures in the neck (carotid sinus) that might influence the reactivity of the animal.

Therapy was instituted promptly after taping of the right leg—one hour after release of the tourniquet. It consisted of the intravenous administration of from 325 to 450 cc. of different fluids introduced into a vein of the foreleg for one hour by the drip method. This was discontinued with the removal of the femoral cannula and the taping of the left leg.

Summary of procedure. 1. Intravenous administration of nembutal 25 mgm./kgm. followed if necessary after several hours with 2–4 mgm./kgm. 2. Application of tourniquets to both hind legs. 3. Release of tourniquet from left leg after five hours followed by passive motion of the leg for a few seconds. 4. Ten minutes later cannulation of left femoral artery for blood pressure record. 5. Release of the tourniquet from the right leg followed by passive motion. 6. One hour later: taping of right leg and institution of intravenous therapy. 7. One hour later: discontinuance of intravenous therapy. 8. Removal of cannula from left femoral artery and closure of wound with sulfathiazole powder dressing. 9. Taping of left leg. 10. Removal of tapes from both legs after 24 hrs.

The animals were protected from cold and kept at about 21°C. Survival beyond 48 hrs. was chosen arbitrarily as the end point but it should be recorded that animals that lived for this period very rarely died without cause quite independent from shock.

Experiments. The experiments may be grouped into the following categories and will be presented in this order. The fundamental procedures are the

same for all groups. Two primary variants only occur. They involve taping of the legs and intravenous therapy. Group I is a control series without taping of legs or therapy of any variety. The legs were taped immediately after release of the tourniquets in group II. In all the others the order of procedure indicated above was followed in detail.

Group I. Control—legs were not taped and no therapy was used. Group II. Legs were taped immediately after release of the tourniquets; on some animals the blood pressure was not recorded. No therapy was used. Group III. Taping alone as indicated in procedure, without supporting therapy. Group IV. Taping and intravenous saline. Group V. Taping and intravenous dog-plasma. Group VI. Taping and intravenous dog-plasma-albumin. Group VII. Taping and intravenous dog-plasma-globulin. Group VIII. Taping and intravenous dialyzed dog-plasma. Group IX. Taping and intravenous pre-

TABLE 1

Shows the survival time of 48 dogs after release of tourniquets applied to both hind legs for 5 hours

SURVIVAL TIME	NUMBER OF DOGS	PERCENTAGE
hours		
1– $1\frac{3}{4}$	6	12.5
2– $2\frac{3}{4}$	12	25.0
3– $3\frac{3}{4}$	8	16.7
4– $4\frac{3}{4}$	6	12.5
5– $5\frac{3}{4}$	3	6.3
7– $7\frac{3}{4}$	3	6.3
11–$11\frac{3}{4}$	3	6.3
13–$13\frac{3}{4}$	2	4.2
15	2	4.2
19	1	2.0
35	1	2.0
48+	1	2.0

Average survival time of 47 animals 5.7 hours.

viously alkalinized dog-plasma. Group X. Taping and intravenous saline plus methylene blue. Group XI. Taping and intravenous dog-plasma-albumin plus methylene blue. Group XII. Taping and intravenous saline plus succinate.

Group I. It should be emphasized that long experience was essential for the development of the procedure outlined above and necessary to secure just those details required for a rapid development of signs of shock that would have a fatal outcome within a few hours without effective therapy. These preliminaries are not included in the tabulated results that follow. Further, the control group was not completed as a unit, but accumulated gradually with small numbers of animals sandwiched in as the therapeutic variants were studied.

This group now comprises 48 dogs included in table 1.

Forty-seven of the 48 dogs died within the arbitrarily chosen limiting time of 48 hrs. Although the survival periods are spread over a larger time interval,

35 or 73 per cent of all animals died during the first $5\frac{3}{4}$ hrs., an average survival time of 3 hrs. Six of these 35 dogs or $12\frac{1}{2}$ per cent died within $1\frac{3}{4}$ hrs. after release of the tourniquet, too rapidly for the institution of therapy. The impression grew that this group with rapid fall of blood pressure to shock level within an hour more or less also developed early bradycardia and complete irregularity far more frequently than those that survived for a longer time.

When these 6 dogs or $12\frac{1}{2}$ per cent are discarded, there remain 29 animals, or 60.5 per cent, that survived 2 to $5\frac{3}{4}$ hrs. after the release of the tourniquets. The blood pressure in these began to fall promptly but did not reach the low level so quickly. It averaged 70–85 mm. Hg after 1 or $1\frac{1}{4}$ hrs. and without therapy the pressure continued to fall.

The remaining 12 animals or 25 per cent had a longer survival period. The large majority of these showed a more gradual drop in blood pressure.

Group II includes 9 animals whose legs were taped immediately on release of the tourniquets from the hind legs. Three animals survived indefinitely. The average survival time for the remaining six was 32+ hrs., the shortest 16 hrs.

There can be little question that immediate taping of the legs after release of the tourniquets plays an important part in the length of survival of the animal. This is in accord with findings of Duncan and Blalock (5).

Group III comprises 9 animals whose hind legs were taped as indicated under procedure. The survival time as compared with that of group I where the legs were not taped is not significantly changed. It averaged $5\frac{2}{3}$ hrs., the spread for individual dogs approximated that of the control group closely and no beneficial effects can be attributed to this procedure alone.

Group IV includes ten animals whose legs were taped after the release of the tourniquets in accordance with the adopted procedure and who received saline intravenously in amounts equal to about 4 per cent of the body weight.

Only one of these ten animals survived indefinitely. It is of interest to note that the shortest survival time was 5 hrs., and the average 12+ hrs. as compared to the control group in which the shortest time was less than 1 hr. and the average 5.7 hrs.

The fall in blood pressure often was marked within the first hour after the release of the tourniquets. In some instances it continued to fall, in others it maintained the same level, while in still others it rose slightly during the infusion. Moreover, these variations in the blood pressure were not related to the survival time.

Group V includes 18 animals that received freshly prepared, pooled, citrated, dog plasma one hour after release of the tourniquets on the hind legs. Table 2 indicates the amount of plasma infusion—in general accord with the body weight—and the fate of the dog.

Only four of the 18 dogs failed to survive the arbitrary figure of 48 hrs. Three of these manifested changes at autopsy that might well have been expressions of other important causes than shock as contributing to their death. The other 14 animals survived the 48 hr. limiting period and may well have lived on indefinitely. This is the more remarkable since the cannulated and tourniquetted

left leg in contrast to the right developed dry gangrene with time in a number of the dogs and provided an excellent nidus for infection.

The value of the plasma began to be manifested after 100± cc. had been infused. Although emphasis can not be laid on this observation the blood pressure began to rise; the cardiac action, often slightly irregular preceding infusion, became stronger and regular; the anesthesia became increasingly less and usually no longer evident by the time the infusion was discontinued. In the next four hours the dogs sat up and took water when this was offered. The record is typical.

TABLE 2

The influence of dog-plasma infusion on the survival time

DOG NO.	WEIGHT	TOTAL AMOUNT OF DOG PLASMA USED	SURVIVAL TIME	REMARKS
	kgm.	*cc.*	*hours*	
1	12	350	∞	
2	10.9	360	∞	
3	12.3	360	∞	
4	9.1	300	∞	
5	9.5	340	∞	
6	12	300	∞	
7	11.4	390	25	Fulminating infection of leg with cl. Welchii
8	13.5	430	∞	
9	9.1	400	38	Pneumonia
10	9.1	400	∞	
11	10.9	390	∞	
12	9.1	350	15	Hemorrhages of the myocardium involving His bundle
13	6.1	350	∞	
14	7.5	490	∞	
15	11.4	400	17	No lesions found at autopsy
16	6.8	350	∞	
17	7.7	400	∞	
18	8.4	350	∞	

Group VI includes 12 dogs that received dog-plasma-albumin one hour after the release of the tourniquets. The plasma-albumin was prepared by saturation of the plasma with ammonium sulfate after the globulins had been removed by half saturation with the same agent. The ammonium sulfate albumin-precipitate was dialyzed first against tap water for one day at room temperature, then against distilled water for two days and finally against saline for two days, both at +4°C. Only traces of ammonium sulfate were detectable after final dialysis. The protein of the albumin solution was determined by the Kjeldahl method and brought to a content of 3.9 to 4.7 per cent to compare with the

colloid osmotic pressure of the plasma.[2] Table 3 shows the weight of the animals, the per cent of albumin and the amount of the infusion fluid as well as the result of the experiment. Only one of these 12 dogs survived for 46 hrs.—one for 24 hrs. and all the rest less than 17 hrs. The average was 16 hrs., 4 hrs. longer than those that were infused with saline. The group includes several animals in which the blood pressure had only fallen slightly, to 85 or 90 mm. Hg when the infusion was started, and had been maintained at this level during

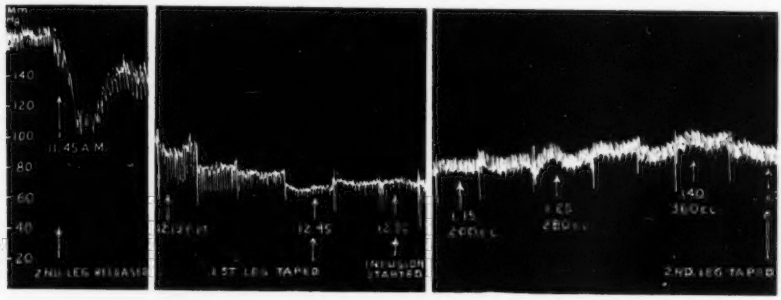

Fig. 1. Blood pressure changes following the release of tourniquets occluding the hind legs of dog 2 of group 5, table 2. Therapy: Taping of legs plus dog plasma infusions.

TABLE 3

The influence of dog-plasma-albumin infusion on the survival time

DOG NO.	WEIGHT	PERCENTAGE OF ALBUMIN SOL. USED	TOTAL AMOUNT OF ALBUMIN SOL. USED	SURVIVAL TIME
	kgm.		*cc.*	*hours*
1	9.5	4.45	340	15
2	14.5	4.45	350	24
3	11.4	4.45	540	14
4	16.4	4.75	400	8
5	13.6	4.75	460	14
6	8.2	4.75	320	14
7	13.6	3.9	480	14
8	13.6	3.9	470	12
9	14.1	3.9	470	46
10	14.1	4.0	400	4
11	10.0	4.0	350	17
12	6.8	4.0	275	13

the following hour while the record was in progress. This is illustrated in the curve of dog 6, table 3.

Group VII. The nine animals in this group received infusions of dog-plasma-globulin after the release of the tourniquets. The globulin was prepared by half saturation with ammonium sulfate; the resulting precipitate was dialyzed

[2] It is assumed that at equal molar concentrations the colloid-osmotic pressure (6) of albumin is at least 2.4 times larger than of globulin.

against tap water for a day at room temperature and then against 2 per cent saline for three days at 4°C. The protein content of the solution was ascertained by aid of the Kjeldahl method and brought to 4.75–5 per cent concentration with 1 per cent saline. One of these nine animals survived indefinitely, the remaining eight died in from 4–22 hrs.—an average of 8.7 hrs. This compares unfavorably with albumin with a survival period of 16+ hrs. and even with saline with 12+ hrs.

Group VIII includes eight animals infused with dialyzed dog-plasma 1 hr. after the release of the tourniquets. The dialysis of pooled dog-plasma was effected in closed cellophane sacs against saline at 4°C. for 8–10 days.

In contrast to undialyzed plasma only one of these eight animals survived indefinitely. The others had an average survival period of 9½ hrs. spread between 5 and 17 hrs. This does not compare favorably even with the results after saline infusion. It indicates clearly that plasma loses some important factor through dialysis as this was carried out.

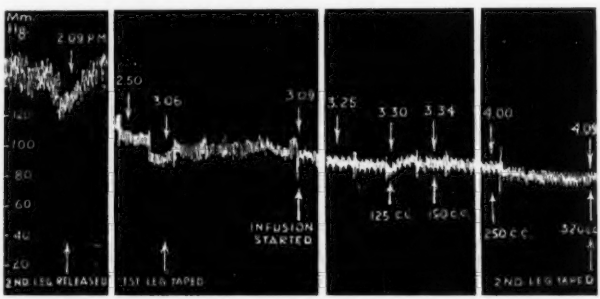

Fig. 2. Blood pressure changes following the release of tourniquets occluding the hind legs of dog 6 of group 6, table 3. Therapy: Taping of legs plus infusion with dog plasma albumin.

Group IX includes 8 dogs infused with dog plasma. This was alkalinized with sodium hydroxide to pH 9.9–10.9, allowed to remain so at +4°C. for 3 days and then return to pH 7.3 with hydrochloric acid. This plasma was not comparably efficacious in saving the lives of the animals. Two dogs did survive indefinitely but the other 6 all died within 19 hrs., an average of 11⅓ hrs. One of the survivors received plasma that had been alkalinized to pH 9.9 and one to pH 10.9. This indicated that the unfavorable influences exerted by the alkali on the plasma are manifest at the lower pH level.

Group X consists of 9 dogs infused with saline supplemented with from two to three intravenous injections of 5 cc. of 0.5 per cent methylene blue in saline during the course of the 1 hr. of therapy.

The use of methylene blue was based upon the hypothesis that interference with tissue respiration may result in changes against which the hydrogen receptor properties this dye offered might have advantages. The effect it exerts on blood pressure also was deemed desirable even if the manner in which this is

brought about is not clear. In accord with previous experience it was found that methylene blue injection was followed invariably by a rise in blood pressure. If 50 mgm. of the dye are injected rapidly in 0.5 per cent concentration, the rise is acute and varies between 10 and 30 mm. Hg. When the injection is repeated

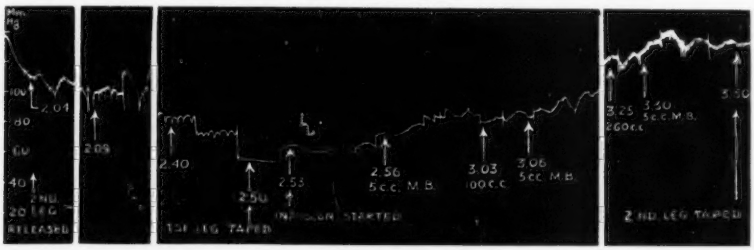

Fig. 3. Blood pressure changes following the release of tourniquets occluding the hind legs of dog 13 of group II, table 4. Therapy: Taping of legs plus infusion of dog plasma albumin and injection of methylene blue.

TABLE 4

The influence of infusion with dog-plasma-albumin plus methylene blue on the survival time

DOG NO.	WEIGHT	TOTAL AMOUNT OF ALBUMIN SOL. USED	AMOUNT OF METHYLENE BLUE	SURVIVAL TIME
	kgm.	*cc.*	*mgm.*	*hours*
1	6.8	275	50	∞
2	7.7	290	70	∞
3	6.4	250	60	∞
4	6.6	270	55	5
5	15.0	410	50	∞
6	11.8	375	75	13
7	15.0	390	90	8
8	11.4	380	90	16
9	6.8	300	60	∞
10	8.0	350	75	40
11	7.3	350	65	∞
12	8.6	325	75	6
13	8.0	325	75	∞
14	6.8	450	75	12
15	7.7	400	75	38
16	10.0	440	80	7
17	7.7	400	70	22
18	8.6	400	75	19
19	7.7	300	40	∞

five or ten minutes later the response is of the same order of magnitude. Only one dog survived and this animal did not have a marked fall in pressure before therapy was started. This probably would have occurred had there been further delay in treatment. The other seven dogs had a spread of from 3 to 36 hrs. in survival time.

Group XI. These animals were subjected to the same procedures used in the preceding group with one exception. They were infused with dog-plasma-albumin instead of saline. The methylene blue injections were followed by the response already detailed. This is illustrated in figure 3. The blood pressure response following the injection of methylene blue though invariably

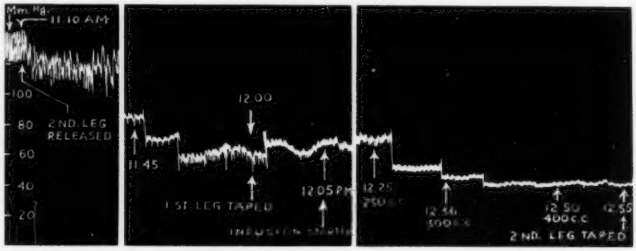

Fig. 4. Blood pressure changes gollowing the release of tourniquets occluding the hind legs of dog 11 of group 12, table 5. Therapy: Taping of legs plus infusion of saline containing succinate. Note the lack of immediate influence of the infusion on the blood pressure—and the survival of the animal.

TABLE 5

The influence of infusion with saline plus succinate on the survival time

DOG NO.	WEIGHT	TOTAL AMOUNT OF SALINE USED	SUCCINATE USED	SURVIVAL TIME
	kgm.	*cc.*	*mgm.*	*hours*
1	7.7	400	210	10
2	9.5	400	210	∞
3	8.6	400	150	∞
4	11.4	400	225	14
5	9.8	400	225	10
6	8.0	400	600	∞
7	8.2	400	600	∞
8	11.4	375	600	6
9	10.5	400	600	13
10	9.1	400	600	14
11	9.5	400	600	∞
12	8.2	400	600	16
13	8.4	400	1200	10
14	10.5	400	1200	12
15	10.9	500	1200	∞
16	8.2	500	1200	∞

present is not an absolute indication of the outcome of the experiment. Eight of the 19 dogs did survive, two of the remainder lived 38 and 40 hrs. respectively (table 4).

Group XII includes 16 dogs infused with saline supplemented with sodium succinate adjusted with hydrochloric acid to a pH of 7.3.

Succinate was used because of the recognized influence it exerts on the respira-

tion of surviving tissue. Whether it acts as a substrate bridging the interval when the tissue can not supply the necessary materials for oxidation or whether it functions as catalyst in the respiratory chain is still undecided.[3] Seven of these 16 animals survived.

The blood pressure records of dogs 11 and 14 contrast remarkably and this is particularly interesting as dog 11, whose blood pressure had fallen from 140 mm. Hg before the release of the tourniquets to less than 50 mm. Hg when therapy was discontinued, recovered completely and survived, while dog 14 whose pressure was 134 mm. Hg just before release of the tourniquets, fell to 64 mm. Hg within 50 min. and then again rose to the pre-release level before therapy was discontinued, died within 12 hrs.

The remarkable effect of the succinate-reinforced saline infusion on the heart should receive special mention. The cardiac contractions in most instances rapidly grew stronger and more regular as the infusion progressed and then a few hours after therapy was discontinued the behavior of the dogs in general was much more like those treated with plasma than after other therapies used, including albumin. Many sat up in their cages, accepted fluid and in general responded to the influences of their environment (table 5).

SUMMARY

1. A procedure is described that leads to "shock" with its characteristic manifestations of drop in blood pressure, hemoconcentration, loss of plasma protein and further in the death of 97 to 98 per cent of the dogs subjected to it. To attain this result the tourniquets must be applied not only to shut off the circulation of the hind legs but tightly enough actually to damage the underlying soft tissues.

2. When the legs are taped before or immediately after release of the tourniquets the incidence of shock is materially reduced and even when signs develop many animals ultimately survive.

3. The processes active in the damaged tissues during the first hour after the restoration of the circulation, including the local loss of fluid, are adequate to initiate fatal shock. Interference with these by taping the legs one hour after the release of the tourniquets is followed by a slightly longer survival period but not by increased recovery.

4. Therapy may be instituted at the end of one hour after the release of the tourniquets on the hind legs. At this time the animals are divisible into three groups on the basis of the severity of their symptoms. Twelve and a half per cent of the group are in an agonal state; 25 per cent do not yet show adequate signs of shock—the remaining 60.5 per cent have a marked fall in blood pressure and without treatment would die within a few hours. This latter group was chosen for therapy.

5. Restoration of the fluid content of the blood by intravenous infusion of

[3] Acknowledgment is gratefully made to Dr. Ephraim Shorr, Cornell Medical School, New York, for his advice concerning the amount of succinate and other valuable suggestions.

saline increases the survival period but leads to recovery only in one out of 10 animals.

6. Intravenous infusion of dog-plasma-albumin of a colloid-osmotic pressure equivalent at least to that of the total plasma further prolongs the survival period but does not influence recovery.

7. Globulin preparations of 5 per cent concentration from dog-plasma are not as effective as saline.

8. Citrated dog-plasma led to complete recovery in 14 out of 18 dogs. The four animals that died had a long survival period and 3 of them showed adequate other cause than shock for death.

9. Dog-plasma dialyzed as described is no longer effective as a therapeutic agent.

10. Dog-plasma alkalinized and readjusted to the original hydrogen concentration is no longer effective therapeutically.

11. Methylene blue administered intravenously promptly raises the blood pressure of an animal in shock. As a supplement to intravenous saline infusion it neither increases survival time nor percentage recovery.

12. Methylene blue as a supplement to dog-plasma albumin infusion prolongs the survival period and raises recovery rate to 42 per cent.

13. Sodium succinate, injected intravenously, is followed temporarily by more forcible and more regular cardiac action. As a supplement to saline infusion it results in a recovery of 43 per cent of the animals.

14. The beneficial influence of succinate contrasts to the known ineffectiveness of glucose. This may indicate a definite disturbance in the earlier stages of the carbohydrate breakdown. It is not known whether the phosphorus turnover is involved.

15. The therapeutic value of plasma is not fully explained by its colloidal osmotic pressure. The search for labile substances in plasma which restore metabolic processes is indicated.

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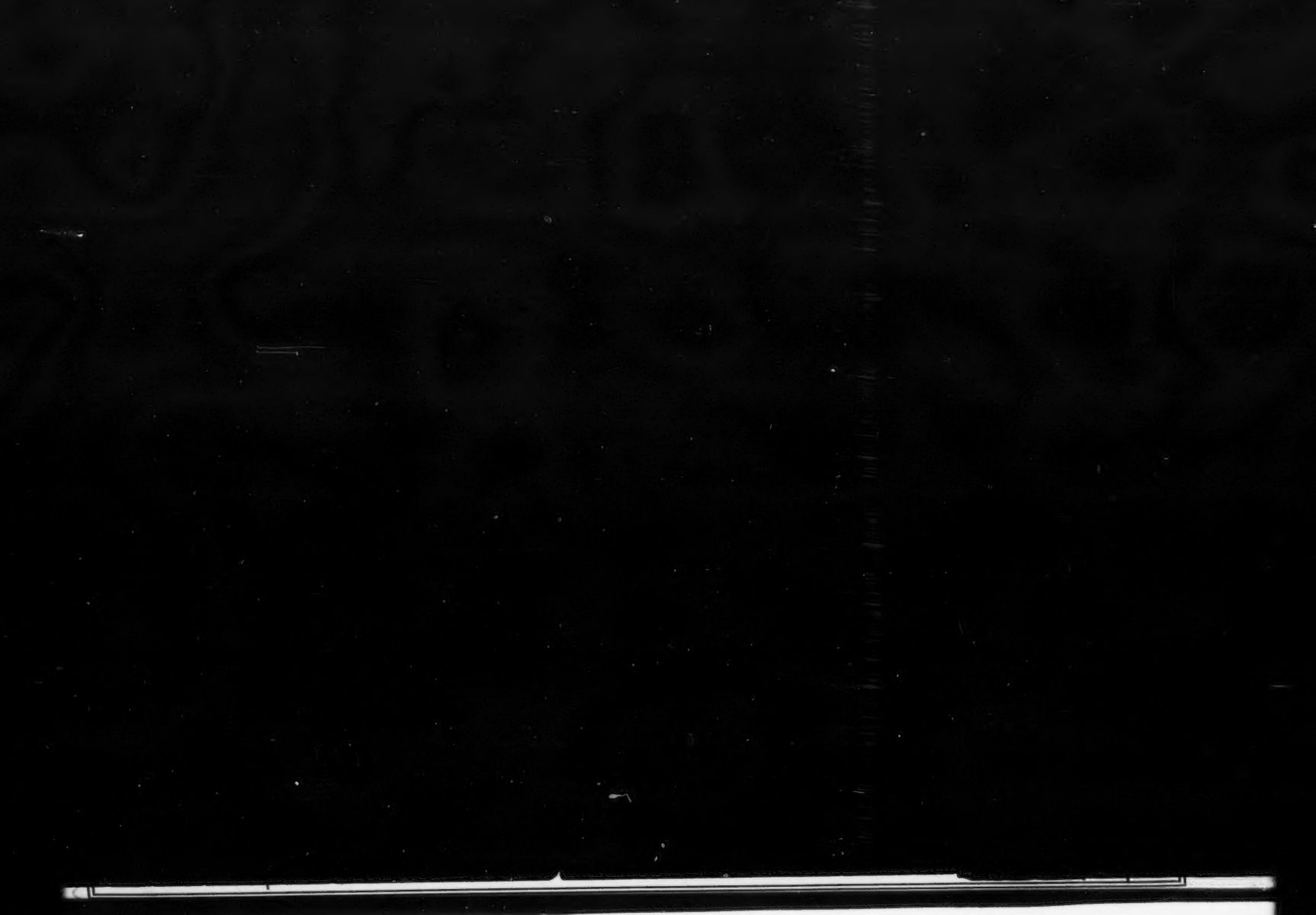
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